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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 : A61K 31/19, 31/71 // (A61K 31/19 A61K 31:20, 31:35, 31:375 A61K 31:07, 31:015)	A1	(11) International Publication Number: WO 93/16690 (43) International Publication Date: 2 September 1993 (02.09.93)

(21) International Application Number: PCT/US93/00260

(22) International Filing Date: 13 January 1993 (13.01.93)

(30) Priority data:
841,342 25 February 1992 (25.02.92) US

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(81) Designated States: AU, CA, JP, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: CYTOPROTECTIVE COMPOSITIONS CONTAINING PYRUVATE AND ANTIOXIDANTS

(57) Abstract

The present invention pertains to cytoprotective compositions for preventing and reducing injury to mammalian cells from a medicament having cytotoxic properties. In one embodiment, the cytoprotective composition comprises (a) pyruvate selected from the group consisting of pyruvic acid, pharmaceutically acceptable salts of pyruvic acid, and mixtures thereof, and (b) an antioxidant. In a second embodiment, the cytoprotective composition comprises pyruvate selected from the group consisting of pyruvic acid, pharmaceutically acceptable salts of pyruvic acid, and mixtures thereof, (b) an antioxidant, and (c) a mixture of saturated and unsaturated fatty acids wherein the fatty acids are those fatty acids required for the repair of cellular membranes and resuscitation of mammalian cells. The cytoprotective compositions may be incorporated into a wide variety of pharmaceutically acceptable carriers to prepare pharmaceutical products. This invention also relates to methods for preparing and using the cytoprotective compositions and the pharmaceutical products in which the cytoprotective compositions may be used.

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CYTOPROTECTIVE COMPOSITIONS CONTAINING PYRUVATE AND ANTIOXIDANTS

BACKGROUND OF THE INVENTION

This application is a continuation-in-part of copending application serial no. 663,500, filed 1 March 1991.

Field of the Invention

This invention pertains to cytoprotective compositions for preventing and reducing injury to mammalian cells from a medicament having cytotoxic properties, and increasing the resuscitation and proliferation rates of the cells. In a first embodiment, the cytoprotective composition comprises (a) pyruvate selected from the group consisting of pyruvic acid, pharmaceutically acceptable salts of pyruvic acid, and mixtures thereof, and (b) an antioxidant. In a second embodiment, the cytoprotective composition comprises pyruvate selected from the group consisting of pyruvic acid, pharmaceutically acceptable salts of pyruvic acid, and mixtures thereof, (b) an antioxidant, and (c) a mixture of saturated and unsaturated fatty acids wherein

the fatty acids are those fatty acids required for the repair of cellular membranes and resuscitation of mammalian cells. The cytoprotective compositions may be incorporated into a wide variety of pharmaceutically acceptable carriers to prepare pharmaceutical products. This invention also relates to methods for preparing and using the cytoprotective compositions and the pharmaceutical products in which the cytoprotective compositions may be used.

Description of the Background

Cancer is a group of neoplastic diseases affecting different organs and systems in the body. A common feature in all cancers is cellular mutation and abnormal and uncontrolled cell growth usually at a rate greater than that of normal body cells. Neither the etiology of cancer nor the manner in which cancer causes death is completely understood.

Significant advances have been made in the chemotherapeutic treatment of cancer. Most anticancer agents act at specific phases of the cell cycle and are therefore only active against cells in the process of division. Although differences in the duration of the cell cycle occur between different types of cells, all cells show a similar pattern during the division process which may be characterized as follows: (1) a presynthetic phase; (2) a DNA synthesis phase; (3) a postsynthetic phase following termination of DNA synthesis; and (4) a mitosis phase, wherein the cell containing a double complement of DNA divides into two daughter cells. Most anti-neoplastic agents act specifically on processes such as the DNA synthesis phase, the transcription phase, or the mitosis phase and are therefore considered cell-cycle specific agents.

A problem with the chemotherapeutic treatment of cancer is that normal cells which proliferate rapidly, such as those in bone marrow, hair follicles, and the gastrointestinal tract, are often damaged or killed by the anti-neoplastic agents. This cytotoxicity problem occurs because the metabolism of cancer cells is similar to that of normal cells and anticancer agents lack specificity for cancer cells. Because most of the metabolic differences between normal and neoplastic cells are quantitative, anticancer drugs are usually employed at or near the toxic range in order to obtain satisfactory therapeutic effects.

When cells are injured or killed as a result of a cytotoxic agent, a cytoprotective step is desirable to protect the cells from the cytotoxic agent, resuscitate the injured cells, and help produce new cells to replace the dead cells. Injured cells require low levels of oxygen in the initial stages of recovery to suppress oxidative damage and higher levels of oxygen in the later stages of recovery to stimulate cellular viability and proliferation.

Stressed and injured mammalian cells are often exposed to activated oxygen species such as superoxide (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radical ($OH^\cdot$), and singlet oxygen ($^1O_2$). *In vivo*, these reactive oxygen intermediates are generated by cells in response to aerobic metabolism, catabolism of drugs and other xenobiotics, ultraviolet and x-ray radiation, and the respiratory burst of phagocytic cells (such as white blood cells) to kill invading bacteria. Hydrogen peroxide, for example, is produced during respiration of most living organisms especially by stressed and injured cells.

These active oxygen species can injure and kill cells. An important example of such damage is lipid peroxidation which involves the oxidative degradation of

unsaturated lipids. Lipid peroxidation is highly detrimental to membrane structure and function and can cause numerous cytopathological effects. Cells defend against lipid peroxidation by producing radical scavengers such as superoxide dismutase, catalase, and peroxidase. Injured cells have a decreased ability to produce radical scavengers. Excess hydrogen peroxide can react with DNA to cause backbone breakage, produce mutations, and alter and liberate bases. Hydrogen peroxide can also react with pyrimidines to open the 5, 6-double bond, which reaction inhibits the ability of pyrimidines to hydrogen bond to complementary bases, Hallaender et al. (1971). Such oxidative biochemical injury can result in the loss of cellular membrane integrity, reduced enzyme activity, changes in transport kinetics, changes in membrane lipid content, and leakage of potassium ions, amino acids, and other cellular material.

Antioxidants have been shown to inhibit damage associated with active oxygen species. For example, pyruvate and other *alpha*-ketoacids have been reported to react rapidly and stoichiometrically with hydrogen peroxide to protect cells from cytolytic effects, O'Donnell-Tormey et al., J. Exp. Med., 165, pp. 500-514 (1987).

United States patents nos. 3,920,835, 3,984,556, and 3,988,470, all issued to Van Scott et al., disclose methods for treating acne, dandruff, and palmar keratosis, respectively, which consist of applying to the affected area a topical composition comprising from about 1% to about 20% of a lower aliphatic compound containing from two to six carbon atoms selected from the group consisting of *alpha*-hydroxyacids, *alpha*-ketoacids and esters thereof, and 3-hydroxybutryic acid in a pharmaceutically acceptable carrier. The aliphatic compounds include pyruvic acid and lactic acid.

United States patents nos. 4,105,783 and 4,197,316, both issued to Yu et al., disclose a method and composition, respectively, for treating dry skin which consists of applying to the affected area a topical composition comprising from about 1% to about 20% of a compound selected from the group consisting of amides and ammonium salts of *alpha*-hydroxyacids, *beta*-hydroxyacids, and *alpha*-ketoacids in a pharmaceutically acceptable carrier. The compounds include the amides and ammonium salts of pyruvic acid and lactic acid.

United States patent no. 4,234,599, issued to Van Scott et al., discloses a method for treating actinic and nonactinic skin keratoses which consists of applying to the affected area a topical composition comprising an effective amount of a compound selected from the group consisting of *alpha*-hydroxyacids, *beta*-hydroxyacids, and *alpha*-ketoacids in a pharmaceutically acceptable carrier. The acidic compounds include pyruvic acid and lactic acid.

United States patent no. 4,294,852, issued to Wildnauer et al., discloses a composition for treating skin which comprises the *alpha*-hydroxyacids, *beta*-hydroxyacids, and *alpha*-ketoacids disclosed above by Van Scott et al. in combination with C_3-C_8 aliphatic alcohols.

United States patent no. 4,663,166, issued to Veech, discloses an electrolyte solution which comprises a mixture of L-lactate and pyruvate in a ratio from 20:1 to 1:1, respectively, or a mixture of D-*beta*-hydroxybutyrate and acetoacetate, in a ratio from 6:1 to 0.5:1, respectively.

Sodium pyruvate has been reported to reduce the number of erosions, ulcers, and hemorrhages on the gastric mucosa in guinea pigs and rats caused by acetylsalicylic acid. The analgesic and antipyretic

properties of acetylsalicylic acid were not impaired by sodium pyruvate, Puschmann, Arzneimittelforschung, 33, pp. 410-415 and 415-416 (1983).

Pyruvate has been reported to exert a positive inotropic effect in stunned myocardum, which is a prolonged ventricular dysfunction following brief periods of coronary artery occlusions which does not produce irreversible damage, Mentzer et al., Ann. Surg., 209, pp. 629-633 (1989).

Pyruvate has been reported to produce a relative stabilization of left ventricular pressure and work parameter and to reduce the size of infarctions. Pyruvate improves resumption of spontaneous beating of the heart and restoration of normal rates and pressure development, Bunger et al., J. Mol. Cell. Cardiol., 18, pp. 423-438 (1986), Mochizuki et al., J. Physiol. (Paris), 76, pp. 805-812 (1980), Regitz et al., Cardiovasc. Res., 15, pp. 652-658 (1981), Giannelli et al., Ann. Thorac. Surg., 21, pp. 386-396 (1976).

Sodium pyruvate has been reported to act as an antagonist to cyanide intoxification (presumably through the formation of a cyanohydrin) and to protect against the lethal effects of sodium sulfide and to retard the onset and development of functional, morphological, and biochemical measures of acrylamide neuropathy of axons, Schwartz et al., Toxicol. Appl. Pharmacol., 50, pp. 437-442 (1979), Sabri et al., Brain Res., 483, pp. 1-11 (1989).

A chemotherapeutic cure of advanced L1210 leukemia has been reported using sodium pyruvate to restore abnormally deformed red blood cells to normal. The deformed red blood cells prevented adequate drug delivery to tumor cells, Cohen, Cancer Chemother. Pharmacol., 5, pp. 175-179 (1981).

Primary cultures of heterotopic tracheal transplant exposed *in vivo* to 7, 12-dimethyl-benz(a)anthracene were reported to be successfully maintained in enrichment medium supplemented with sodium pyruvate along with cultures of interleukin-2 stimulated peripheral blood lymphocytes, and plasmacytomas and hybridomas, pig embryos, and human blastocysts, Shacter, J. Immunol. Methods, 99, pp. 259-270 (1987), Marchok et al., Cancer Res., 37, pp. 1811-1821 (1977), Davis, J. Reprod. Fertil. Suppl., 33, pp. 115-124 (1985), Okamoto et al., No To Shinkei, 38, pp. 593-598 (1986), Cohen et al., J. In Vitro Fert. Embryo Transfer, 2, pp. 59-64 (1985).

United States patents nos. 4,158,057, 4,351,835, 4,415,576, and 4,645,764, all issued to Stanko, disclose methods for preventing the accumulation of fat in the liver of a mammal due to the ingestion of alcohol, for controlling weight in a mammal, for inhibiting body fat while increasing protein concentration in a mammal, and for controlling the deposition of body fat in a living being, respectively. The methods comprise administering to the mammal a therapeutic mixture of pyruvate and dihydroxyacetone, and optionally riboflavin. United States patent no. 4,548,937, issued to Stanko, discloses a method for controlling the weight gain of a mammal which comprises administering to the mammal a therapeutically effective amount of pyruvate, and optionally riboflavin. United States patent no. 4,812,479, issued to Stanko, discloses a method for controlling the weight gain of a mammal which comprises administering to the mammal a therapeutically effective amount of dihydroxyacetone, and optionally riboflavin and pyruvate.

Rats fed a calcium-oxalate lithogenic diet including sodium pyruvate were reported to develop fewer urinary calculi (stones) than control rats not given

sodium pyruvate, Ogawa et al., Hinyokika Kiyo, 32, pp. 1341-1347 (1986).

United States patent no. 4,521,375, issued to Houlsby, discloses a method for sterilizing surfaces which come into contact with living tissue. The method comprises sterilizing the surface with aqueous hydrogen peroxide and then neutralizing the surface with pyruvic acid.

United States patent no. 4,416,982, issued to Tauda et al., discloses a method for decomposing hydrogen peroxide by reacting the hydrogen peroxide with a phenol or aniline derivative in the presence of peroxidase.

United States patent no. 4,696,917, issued to Lindstrom et al., discloses an eye irrigation solution which comprises Eagle's Minimum Essential Medium with Earle's salts, chondroitin sulfate, a buffer solution, 2-mercaptoethanol, and a pyruvate. The irrigation solution may optionally contain ascorbic acid and *alpha*-tocopherol. United States patent no. 4,725,586, issued to Lindstrom et al., discloses an irrigation solution which comprises a balanced salt solution, chondroitin sulfate, a buffer solution, 2-mercaptoethanol, sodium bicarbonate or dextrose, a pyruvate, a sodium phosphate buffer system, and cystine. The irrigation solution may optionally contain ascorbic acid and *gamma*-tocopherol.

United States patent no. 3,887,702 issued to Baldwin, discloses a composition for treating fingernails and toenails which consists essentially of soybean oil or sunflower oil in combination with Vitamin E.

United States patent no. 4,847,069, issued to Bissett et al., discloses a photoprotective composition comprising (a) a sorbohydroxamic acid, (b) an anti-inflammatory agent selected from steroidal anti-inflammatory agents and a natural anti-inflammatory

agent, and (c) a topical carrier. Fatty acids may be present as an emollient. United States patent no. 4,847,071, issued to Bissett et al., discloses a photoprotective composition comprising (a) a tocopherol or tocopherol ester radical scavenger, (b) an anti-inflammatory agent selected from steroidal anti-inflammatory agents and a natural anti-inflammatory agent, and (c) a topical carrier. United States patent no. 4,847,072, issued to Bissett et al., discloses a topical composition comprising not more than 25% tocopherol sorbate in a topical carrier.

United States patent no. 4,533,637, issued to Yamane et al., discloses a culture medium which comprises a carbon source, a nucleic acid source precursor, amino acids, vitamins, minerals, a lipophilic nutrient, and serum albumin, and cyclodextrins. The lipophilic substances include unsaturated fatty acids and lipophilic vitamins such as Vitamin A, D, and E. Ascorbic acid may also be present.

United Kingdom patent application no. 2,196,348A, to Kovar et al., discloses a synthetic culture medium which comprises inorganic salts, monosaccharides, amino acids, vitamins, buffering agents, and optionally sodium pyruvate adding magnesium hydroxide or magnesium oxide to the emulsion. The oil phase may include chicken fat.

United States patent no. 4,284,630, issued to Yu et al., discloses a method for stabilizing a water-in-oil emulsion which comprises adding magnesium hydroxide or magnesium oxide to the emulsion. The oil phase may include chicken fat.

Preparation-H™ has been reported to increase the rate of wound healing in artificially created rectal ulcers. The active ingredients in Preparation-H™ are skin respiratory factor and shark liver oil,

Subramanyam et al., Digestive Diseases and Sciences, 29, pp. 829-832 (1984).

The addition of sodium pyruvate to bacterial and yeast systems has been reported to inhibit hydrogen peroxide production, enhance growth, and protect the systems against the toxicity of reactive oxygen intermediates. The unsaturated fatty acids and saturated fatty acids contained within chicken fat enhanced membrane repair and reduced cytotoxicity. The antioxidants glutathione and thioglycollate reduced the injury induced by oxygen radical species, Martin, Ph.D. thesis, (1987-89).

United States patent no. 4,615,697, issued to Robinson, discloses a controlled release treatment composition comprising a treating agent and a bioadhesive agent comprising a water-swellable but water-insoluble, fibrous cross-linked carboxy-functional polymer.

European patent application no. 0410696A1, to Kellaway et al., discloses a mucoadhesive delivery system comprising a treating agent and a polyacrylic acid cross-linked with from about 1% to about 20% by weight of a polyhydroxy compound such as a sugar, cyclitol, or lower polyhydric alcohol.

While the above therapeutic compositions are reported to inhibit the production of reactive oxygen intermediates, none of the above compositions are entirely satisfactory cytoprotective compositions. None of the compositions has the ability to simultaneously decrease cellular levels of hydrogen peroxide production, increase cellular resistance to cytotoxic agents, increase rates of cellular proliferation, and increase cellular viability to protect and resuscitate mammalian cells. The present invention provides such improved therapeutic cytoprotective compositions without the

disadvantages characteristic of previously known compositions.

SUMMARY OF THE INVENTION

The present invention pertains to cytoprotective compositions for preventing and reducing injury to mammalian cells from a medicament having cytotoxic properties. In a first embodiment, the cytoprotective composition comprises (a) pyruvate selected from the group consisting of pyruvic acid, pharmaceutically acceptable salts of pyruvic acid, and mixtures thereof, and (b) an antioxidant. In a second embodiment, the cytoprotective composition comprises (a) pyruvate selected from the group consisting of pyruvic acid, pharmaceutically acceptable salts of pyruvic acid, and mixtures thereof, (b) an antioxidant, and (c) a mixture of saturated and unsaturated fatty acids wherein the fatty acids are those fatty acids required for the repair of cellular membranes and resuscitation of mammalian cells.

The cytoprotective compositions of the present invention may be administered to cells concurrently with a cytotoxic agent. The cytoprotective compositions may also be administered to cells prior to administration of a cytotoxic anticancer agent to selectively protect non-cancerous cells in the presence of cancerous cells.

The cytoprotective compositions may be incorporated into a wide variety of pharmaceutically acceptable carriers to prepare pharmaceutical products. This invention also relates to methods for preparing and using the cytoprotective compositions and the pharmaceutical products in which the cytoprotective compositions may be used.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1, top portion, is a graph showing the viability of U937 monocytic leukemia tumor cells after 24 hours, as determined by tritiated thymidine incorporation assay, following treatment of the cells with different dosage levels of Doxorubicin. Figure 1, bottom portion, is a graph showing the viability of U937 monocytic leukemia tumor cells after 24 hours, as determined by exclusion of the vital dye trypan blue assay, following treatment of the cells with different dosage levels of Doxorubicin.

Figure 2 is a graph showing the viability of U937 monocytic leukemia tumor cells after 1 hour, as determined by exclusion of the vital dye trypan blue assay, following treatment of the cells with different dosage levels of Doxorubicin.

Figure 3 is a graph showing the viability of U937 monocytic leukemia tumor cells after 24 hours, as determined by tritiated thymidine incorporation assay, following treatment of the cells with the cytoprotective components of the present invention, alone and in combinations, at different dosage levels.

Figure 4, top portion, is a graph showing the viability of U937 monocytic leukemia tumor cells in a wash-out study, as determined by tritiated thymidine incorporation assay, after 24 hour pretreatment of the cells with 5 mM sodium pyruvate followed by administration of different dosage levels of Doxorubicin. Figure 4, bottom portion, is a graph showing the viability of peripheral blood monocytes in a wash-out study, as determined by tritiated thymidine incorporation assay, after 24 hour pretreatment of the cells with 5 mM

sodium pyruvate followed by administration of different dosage levels of Doxorubicin.

Figure 5, top portion, is a graph showing the viability of U937 monocytic leukemia tumor cells in a wash-out study, as determined by tritiated thymidine incorporation assay, after 24 hour pretreatment of the cells with 0.5% fatty acids followed by administration of different dosage levels of Doxorubicin. Figure 5, bottom portion, is a graph showing the viability of peripheral blood monocytes in a wash-out study, as determined by tritiated thymidine incorporation assay, after 24 hour pretreatment of the cells with 0.5% fatty acids followed by administration of different dosage levels of Doxorubicin.

Figure 6, top portion, is a graph showing the viability of U937 monocytic leukemia tumor cells in a wash-out study, as determined by tritiated thymidine incorporation assay, after 24 hour pretreatment of the cells with 10 U Vitamin E followed by administration of different dosage levels of Doxorubicin. Figure 6, bottom portion, is a graph showing the viability of peripheral blood monocytes in a wash-out study, as determined by tritiated thymidine incorporation assay, after 24 hour pretreatment of the cells with 10 U Vitamin E followed by administration of different dosage levels of Doxorubicin.

Figure 7, top portion, is a graph showing the viability of U937 monocytic leukemia tumor cells in a wash-out study, as determined by tritiated thymidine incorporation assay, after 24 hour pretreatment of the cells with 50 U Vitamin E followed by administration of different dosage levels of Doxorubicin. Figure 7, bottom portion, is a graph showing the viability of peripheral blood monocytes in a wash-out study, as determined by tritiated thymidine incorporation assay, after 24 hour pretreatment of the cells with 50 U Vitamin E followed by administration of different dosage levels of Doxorubicin.

Figure 8, top portion, is a graph showing the viability of U937 monocytic leukemia tumor cells in a wash-out study, as determined by tritiated thymidine incorporation assay, after 24 hour pretreatment of the cells with 5 mM sodium pyruvate and 0.5% fatty acids followed by administration of different dosage levels of Doxorubicin. Figure 8, bottom portion, is a graph showing the viability of peripheral blood monocytes in a wash-out study, as determined by tritiated thymidine incorporation assay, after 24 hour pretreatment of the cells with 5 mM sodium pyruvate and 0.5% fatty acids followed by administration of different dosage levels of Doxorubicin.

Figure 9, top portion, is a graph showing the viability of U937 monocytic leukemia tumor cells in a wash-out study, as determined by tritiated thymidine incorporation assay, after 24 hour pretreatment of the cells with 5 mM sodium pyruvate and 10 U Vitamin E followed by administration of different dosage levels of Doxorubicin. Figure 9, bottom portion, is a graph showing the viability of peripheral blood monocytes in a wash-out study, as determined by tritiated thymidine incorporation assay, after 24 hour pretreatment of the cells with 5 mM sodium pyruvate and 10 U Vitamin E followed by administration of different dosage levels of Doxorubicin.

Figure 10, top portion, is a graph showing the viability of U937 monocytic leukemia tumor cells in a wash-out study, as determined by tritiated thymidine incorporation assay, after 24 hour pretreatment of the cells with 5 mM sodium pyruvate and 50 U Vitamin E followed by administration of different dosage levels of Doxorubicin. Figure 10, bottom portion, is a graph showing the viability of peripheral blood monocytes in a wash-out study, as determined by tritiated thymidine incorporation assay, after 24 hour pretreatment of the

cells with 5 mM sodium pyruvate and 50 U Vitamin E followed by administration of different dosage levels of Doxorubicin.

Figure 11, top portion, is a graph showing the viability of U937 monocytic leukemia tumor cells in a wash-out study, as determined by tritiated thymidine incorporation assay, after 24 hour pretreatment of the cells with 10 U Vitamin E and 0.5% fatty acids followed by administration of different dosage levels of Doxorubicin. Figure 11, bottom portion, is a graph showing the viability of peripheral blood monocytes in a wash-out study, as determined by tritiated thymidine incorporation assay, after 24 hour pretreatment of the cells with 10 U Vitamin E and 0.5% fatty acids followed by administration of different dosage levels of Doxorubicin.

Figure 12, top portion, is a graph showing the viability of U937 monocytic leukemia tumor cells in a wash-out study, as determined by tritiated thymidine incorporation assay, after 24 hour pretreatment of the cells with 50 U Vitamin E and 0.5% fatty acids followed by administration of different dosage levels of Doxorubicin. Figure 12, bottom portion, is a graph showing the viability of peripheral blood monocytes in a wash-out study, as determined by tritiated thymidine incorporation assay, after 24 hour pretreatment of the cells with 50 U Vitamin E and 0.5% fatty acids followed by administration of different dosage levels of Doxorubicin.

Figure 13, top portion, is a graph showing the viability of U937 monocytic leukemia tumor cells in a wash-out study, as determined by tritiated thymidine incorporation assay, after 24 hour pretreatment of the cells with 5 mM sodium pyruvate, 10 U Vitamin E, and 0.5% fatty acids followed by administration of different dosage levels of Doxorubicin. Figure 13, bottom portion,

is a graph showing the viability of peripheral blood monocytes in a wash-out study, as determined by tritiated thymidine incorporation assay, after 24 hour pretreatment of the cells with 5 mM sodium pyruvate, 10 U Vitamin E, and 0.5% fatty acids followed by administration of different dosage levels of Doxorubicin.

Figure 14, top portion, is a graph showing the viability of U937 monocytic leukemia tumor cells in a wash-out study, as determined by tritiated thymidine incorporation assay, after 24 hour pretreatment of the cells with 5 mM sodium pyruvate, 50 U Vitamin E, and 0.5% fatty acids followed by administration of different dosage levels of Doxorubicin. Figure 14, bottom portion, is a graph showing the viability of peripheral blood monocytes in a wash-out study, as determined by tritiated thymidine incorporation assay, after 24 hour pretreatment of the cells with 5 mM sodium pyruvate, 50 U Vitamin E, and 0.5% fatty acids followed by administration of different dosage levels of Doxorubicin.

Figure 15, top portion, is a graph showing the viability of U937 monocytic leukemia tumor cells in a co-culture study, as determined by tritiated thymidine incorporation assay, after 24 hour pretreatment of the cells with 5 mM sodium pyruvate followed by administration of different dosage levels of Doxorubicin. Figure 15, bottom portion, is a graph showing the viability of peripheral blood monocytes in a co-culture study, as determined by tritiated thymidine incorporation assay, after 24 hour pretreatment of the cells with 5 mM sodium pyruvate followed by administration of different dosage levels of Doxorubicin.

Figure 16, top portion, is a graph showing the viability of U937 monocytic leukemia tumor cells in a co-culture study, as determined by tritiated thymidine incorporation assay, after 24 hour pretreatment of the cells with 0.5% fatty acids followed by administration of

different dosage levels of Doxorubicin. Figure 16, bottom portion, is a graph showing the viability of peripheral blood monocytes in a co-culture study, as determined by tritiated thymidine incorporation assay, after 24 hour pretreatment of the cells with 0.5% fatty acids followed by administration of different dosage levels of Doxorubicin.

Figure 17, top portion, is a graph showing the viability of U937 monocytic leukemia tumor cells in a co-culture study, as determined by tritiated thymidine incorporation assay, after 24 hour pretreatment of the cells with 10 U Vitamin E followed by administration of different dosage levels of Doxorubicin. Figure 17, bottom portion, is a graph showing the viability of peripheral blood monocytes in a co-culture study, as determined by tritiated thymidine incorporation assay, after 24 hour pretreatment of the cells with 10 U Vitamin E followed by administration of different dosage levels of Doxorubicin.

Figure 18, top portion, is a graph showing the viability of U937 monocytic leukemia tumor cells in a co-culture study, as determined by tritiated thymidine incorporation assay, after 24 hour pretreatment of the cells with 50 U Vitamin E followed by administration of different dosage levels of Doxorubicin. Figure 18, bottom portion, is a graph showing the viability of peripheral blood monocytes in a co-culture study, as determined by tritiated thymidine incorporation assay, after 24 hour pretreatment of the cells with 50 U Vitamin E followed by administration of different dosage levels of Doxorubicin.

Figure 19, top portion, is a graph showing the viability of U937 monocytic leukemia tumor cells in a co-culture study, as determined by tritiated thymidine incorporation assay, after 24 hour pretreatment of the cells with 5 mM sodium pyruvate and 0.5% fatty acids

followed by administration of different dosage levels of Doxorubicin. Figure 19, bottom portion, is a graph showing the viability of peripheral blood monocytes in a co-culture study, as determined by tritiated thymidine incorporation assay, after 24 hour pretreatment of the cells with 5 mM sodium pyruvate and 0.5% fatty acids followed by administration of different dosage levels of Doxorubicin.

Figure 20, top portion, is a graph showing the viability of U937 monocytic leukemia tumor cells in a co-culture study, as determined by tritiated thymidine incorporation assay, after 24 hour pretreatment of the cells with 5 mM sodium pyruvate and 10 U Vitamin E followed by administration of different dosage levels of Doxorubicin. Figure 20, bottom portion, is a graph showing the viability of peripheral blood monocytes in a co-culture study, as determined by tritiated thymidine incorporation assay, after 24 hour pretreatment of the cells with 5 mM sodium pyruvate and 10 U Vitamin E followed by administration of different dosage levels of Doxorubicin.

Figure 21, top portion, is a graph showing the viability of U937 monocytic leukemia tumor cells in a co-culture study, as determined by tritiated thymidine incorporation assay, after 24 hour pretreatment of the cells with 5 mM sodium pyruvate and 50 U Vitamin E followed by administration of different dosage levels of Doxorubicin. Figure 21, bottom portion, is a graph showing the viability of peripheral blood monocytes in a co-culture study, as determined by tritiated thymidine incorporation assay, after 24 hour pretreatment of the cells with 5 mM sodium pyruvate and 50 U Vitamin E followed by administration of different dosage levels of Doxorubicin.

Figure 22, top portion, is a graph showing the viability of U937 monocytic leukemia tumor cells in a co-

culture study, as determined by tritiated thymidine incorporation assay, after 24 hour pretreatment of the cells with 10 U Vitamin E and 0.5% fatty acids followed by administration of different dosage levels of Doxorubicin. Figure 22, bottom portion, is a graph showing the viability of peripheral blood monocytes in a co-culture study, as determined by tritiated thymidine incorporation assay, after 24 hour pretreatment of the cells with 10 U Vitamin E and 0.5% fatty acids followed by administration of different dosage levels of Doxorubicin.

Figure 23, top portion, is a graph showing the viability of U937 monocytic leukemia tumor cells in a co-culture study, as determined by tritiated thymidine incorporation assay, after 24 hour pretreatment of the cells with 50 U Vitamin E and 0.5% fatty acids followed by administration of different dosage levels of Doxorubicin. Figure 23, bottom portion, is a graph showing the viability of peripheral blood monocytes in a co-culture study, as determined by tritiated thymidine incorporation assay, after 24 hour pretreatment of the cells with 50 U Vitamin E and 0.5% fatty acids followed by administration of different dosage levels of Doxorubicin.

Figure 24, top portion, is a graph showing the viability of U937 monocytic leukemia tumor cells in a co-culture study, as determined by tritiated thymidine incorporation assay, after 24 hour pretreatment of the cells with 5 mM sodium pyruvate, 10 U Vitamin E, and 0.5% fatty acids followed by administration of different dosage levels of Doxorubicin. Figure 24, bottom portion, is a graph showing the viability of peripheral blood monocytes in a co-culture study, as determined by tritiated thymidine incorporation assay, after 24 hour pretreatment of the cells with 5 mM sodium pyruvate, 10 U Vitamin E, and 0.5% fatty acids followed by administration of different dosage levels of Doxorubicin.

Figure 25, top portion, is a graph showing the viability of U937 monocytic leukemia tumor cells in a co-culture study, as determined by tritiated thymidine incorporation assay, after 24 hour pretreatment of the cells with 5 mM sodium pyruvate, 50 U Vitamin E, and 0.5% fatty acids followed by administration of different dosage levels of Doxorubicin. Figure 25, bottom portion, is a graph showing the viability of peripheral blood monocytes in a co-culture study, as determined by tritiated thymidine incorporation assay, after 24 hour pretreatment of the cells with 5 mM sodium pyruvate, 50 U Vitamin E, and 0.5% fatty acids followed by administration of different dosage levels of Doxorubicin.

DETAILED DESCRIPTION OF THE INVENTION

Applicant has discovered cytoprotective compositions for protecting mammalian cells from a medicament having cytotoxic properties by preventing and reducing injury to the cells. Cells treated with the cytoprotective compositions of the present invention show decreased levels of hydrogen peroxide production, increased resistance to cytotoxic agents, increased rates of proliferation, and increased viability. The cytoprotective compositions may be administered to cells concurrently with a cytotoxic agent or the cytoprotective compositions may be administered to cells prior to administration of a cytotoxic anticancer agent to selectively protect non-cancerous cells in the presence of cancerous cells. Because cancerous cells have a rapid metabolism, cancerous cells will rapidly consume the protective cytoprotective composition and will not be protected by the cytoprotective compositions when the chemotherapeutic medicament is subsequently administered.

The term "injured cell" as used herein means a cell which has (a) injured membranes so that transport through the membranes is diminished resulting in an increase in toxins and normal cellular wastes inside the cell and a decrease in nutrients and other components necessary for cellular repair inside the cell, (b) an increase in concentration of oxygen radicals inside the cell because of the decreased ability of the cell to produce antioxidants and enzymes, and (c) damaged DNA, RNA, and ribosomes which must be repaired or replaced before normal cellular functions can be resumed. The term "resuscitation" of injured mammalian cells as used herein means the reversal of cytotoxicity, the stabilization of the cellular membrane, an increase in the proliferation rate of the cell, and/or the normalization of cellular functions such as the secretion of growth factors, hormones, and the like. The term "cytotoxicity" as used herein means a condition caused by a cytotoxic agent that injures the cell. Injured cells do not proliferate because injured cells expend all energy on cellular repair. Aiding cellular repair promotes cellular proliferation.

Epidermal keratinocytic cells and monocytic cells have multiple oxygen generating mechanisms and the degree to which each type of mechanism functions differs in each type of cell. In monocytes, for example, the respiratory bursting process is more pronounced than in epidermal keratinocytes. Hence, the components in the cytoprotective compositions of the present invention may vary depending upon the types of cells involved in the condition being treated.

In a first embodiment, the therapeutic cytoprotective composition for treating mammalian cells, preferably epidermal keratinocytes, comprises (a) pyruvate, (b) an antioxidant, and (c) a mixture of saturated and unsaturated fatty acids.

While not wishing to be bound by theory, applicant believes that pyruvate (or pyruvic acid) can be transported inside a cell where it can act as an antioxidant to neutralize oxygen radicals in the cell. Pyruvate can also be used inside the cell in the citric acid cycle to provide energy to increase cellular viability, and as a precursor in the synthesis of important biomolecules to promote cellular proliferation. In addition, pyruvate can be used in the multifunction oxidase system to reverse cytotoxicity. Antioxidants, especially lipid-soluble antioxidants, can be absorbed into the cell membrane to neutralize oxygen radicals and thereby protect the membrane. The combination of pyruvate inside the cell and an antioxidant in the cellular membrane functions in a synergistic manner to reduce hydrogen peroxide production in the cell to levels lower than can be achieved by use of either type of component alone.

The saturated and unsaturated fatty acids in the present invention are those fatty acids required for the repair of cellular membranes and resuscitation of mammalian cells. Hence, the fatty acids in the cytoprotective composition, which may be in the form of mono-, di-, and/or triglycerides or free fatty acids, are readily available for the repair of injured cells and the production of new cells to replace dead cells. Cells injured by oxygen radicals need to produce unsaturated fatty acids to repair cellular membranes. However, the production of unsaturated fatty acids by cells requires oxygen. Thus, the injured cell needs high levels of oxygen to produce unsaturated fatty acids and at the same time needs to reduce the level of oxygen within the cell to reduce oxidative injury. By providing the cell with the unsaturated fatty acids needed for repair, the need of the cell to produce unsaturated fatty acids is reduced and the need for high oxygen levels is also reduced. The presence of mixtures of saturated and unsaturated fatty acids in the cytoprotective composition significantly

enhances the ability of pyruvate and the antioxidant to inhibit reactive oxygen production. By stabilizing the cellular membrane, unsaturated fatty acids also improve membrane function and enhance pyruvate transport into the cell. By improving the viability of the cells, unsaturated fatty acids also improve the repair of cellular membranes rate of the cells. Hence, the three components in the cytoprotective composition function together in a synergistic manner to prevent and reduce injury to mammalian cells, increase the resuscitation rate of injured cells, and increase the production of new cells.

In a second embodiment, the therapeutic cytoprotective composition for treating mammalian cells, preferably epidermal keratinocytes, comprises (a) pyruvate, (b) lactate, and (c) a mixture of saturated and unsaturated fatty acids. In this embodiment, lactate is employed instead of an antioxidant. Antioxidants react with, and neutralize, oxygen radicals after the radicals are already formed. Lactate, on the other hand, is a component in the cellular feedback mechanism and inhibits the respiratory bursting process to suppress the production of active oxygen species. The combination of pyruvate to neutralize active oxygen species and lactate to suppress the respiratory bursting process functions in a synergistic manner to reduce hydrogen peroxide production in the cell to levels lower than can be achieved by use of either type of component alone. The presence of mixtures of saturated and unsaturated fatty acids in the cytoprotective composition significantly enhances the ability of pyruvate and lactate to inhibit reactive oxygen production. Hence, the three components in the cytoprotective composition in this embodiment function together in a synergistic manner to prevent and reduce injury to mammalian cells, and increase the proliferation and resuscitation rate of the cells.

In a third embodiment, the therapeutic cytoprotective composition for treating mammalian cells, preferably epidermal keratinocytes, comprises (a) an antioxidant, and (b) a mixture of saturated and unsaturated fatty acids. The presence of mixtures of saturated and unsaturated fatty acids in the cytoprotective composition in this embodiment significantly enhances the ability of the antioxidant to inhibit reactive oxygen production. The combination of an antioxidant to neutralize active oxygen species and fatty acids to rebuild cellular membranes and reduce the need of the cell for oxygen functions in a synergistic manner to reduce hydrogen peroxide production in the cell to levels lower than can be achieved by either type of component alone. Hence, the components in the cytoprotective composition in this embodiment function together in a synergistic manner to prevent and reduce injury to mammalian cells, and increase the proliferation and resuscitation rate of the cells.

In a fourth embodiment, the therapeutic cytoprotective composition for treating mammalian cells, preferably monocytes, comprises (a) lactate, (b) an antioxidant, and (c) a mixture of saturated and unsaturated fatty acids. In this embodiment, lactate is employed because the respiratory bursting process is more pronounced in monocytes than in epidermal keratinocytes. The combination of lactate to suppress the respiratory bursting process and an antioxidant to neutralize active oxygen species functions in a synergistic manner to reduce hydrogen peroxide production in the cell to levels lower than can be achieved by either component alone. The presence of mixtures of saturated and unsaturated fatty acids in the cytoprotective composition in this embodiment significantly enhances the ability of lactate and the antioxidant to inhibit reactive oxygen production. Hence, the three components in the cytoprotective composition in this embodiment function together in a synergistic manner to prevent and reduce

injury to mammalian cells, and increase the proliferation and resuscitation rate of the cells.

In a fifth embodiment, the therapeutic cytoprotective composition for treating mammalian cells, preferably epidermal keratinocytes, comprises (a) pyruvate, and (b) an antioxidant. When the therapeutic cytoprotective composition in this embodiment is administered to cells before the cytotoxic agent is administered, the combination of pyruvate inside the cell and the antioxidant in the cellular membrane functions in a synergistic manner to reduce hydrogen peroxide production in the cell and thereby prevent injury to the cell. When injury to the cell is prevented and the cell does not require resuscitation, the mixture of saturated and unsaturated fatty acids need not be employed in the cytoprotective composition. Hence, the two components in the cytoprotective composition in this embodiment function together in a synergistic manner to prevent and reduce injury to mammalian cells.

Accordingly, the combination of ingredients set out in the above embodiments functions together in an enhanced manner to prevent and reduce injury to mammalian cells and to increase the proliferation and resuscitation rate of mammalian cells. The therapeutic effect of the combination of the components in each of the above embodiments is markedly greater than that expected by the mere addition of the individual therapeutic components. Hence, applicant's therapeutic cytoprotective compositions have the ability to decrease intracellular levels of hydrogen peroxide production, increase cellular resistance to cytotoxic agents, increase rates of cellular proliferation, and increase cellular viability.

The cells which may be treated with the cytoprotective compositions in the present invention are mammalian cells. Although applicant will describe the present cytoprotective compositions as useful for

treating mammalian epidermal keratinocytes and mammalian monocytes, applicant contemplates that all mammalian cells which may be protected or resuscitated by applicant's cytoprotective compositions may be used in the present invention. Keratinocytes are representative of normal mammalian cells and are the fastest proliferating cells in the body. The correlation between the reaction of keratinocytes to injury and therapy and that of mammalian cells in general is very high. Monocytes are representative of specialized mammalian cells such as the white blood cells in the immune system and the organ cells in liver, kidney, heart, and brain. The mammalian cells may be treated *in vivo* and *in vitro*.

Epidermal keratinocytes are the specialized epithelial cells of the epidermis which synthesize keratin, a scleroprotein which is the principal constituent of epidermis, hair, nails, horny tissue, and the organic matrix of the enamel of teeth. Mammalian epidermal keratinocytes constitute about 95% of the epidermal cells and together with melanocytes form the binary system of the epidermis. In its various successive stages, epidermal keratinocytes are also known as basal cells, prickle cells, and granular cells.

Monocytes are mononuclear phagocytic leukocytes which undergo respiratory bursting and are involved in reactive oxygen mediated damage within the epidermis. Leukocytes are white blood cells or corpuscles which may be classified into two main groups: granular leukocytes (granulocytes) which are leukocytes with abundant granules in the cytoplasm and nongranular leukocytes (nongranulocytes) which are leukocytes without specific granules in the cytoplasm and which include the lymphocytes and monocytes. Phagocyte cells are cells which ingest microorganisms or other cells and foreign particles. Monocytes are also known as large mononuclear leukocytes, and hyaline or transitional leukocytes.

Pyruvic acid (2-oxopropanoic acid, *alpha*-ketopropionic acid, $CH_3COCOOH$) or pyruvate (at physiological pH) is a fundamental intermediate in protein and carbohydrate metabolism and in the citric acid cycle. The citric acid cycle (tricarboxylic acid cycle, Kreb's cycle) is the major reaction sequence which executes the reduction of oxygen to generate adenosine triphosphate (ATP) by oxidizing organic compounds in respiring tissues to provide electrons to the transport system. Acetyl coenzyme A ("active acetyl") is oxidized in this process and is thereafter utilized in a variety of biological processes and is a precursor in the biosynthesis of many fatty acids and sterols. The two major sources of acetyl coenzyme A are derived from the metabolism of glucose and fatty acids. Glycolysis consists of a series of transformations wherein each glucose molecule is transformed in the cellular cytoplasm into two molecules of pyruvic acid. Pyruvic acid may then enter the mitochondria where it is oxidized by coenzyme A in the presence of enzymes and cofactors to acetyl coenzyme A. Acetyl coenzyme A can then enter the citric acid cycle.

In muscle, pyruvic acid (derived from glycogen) is reduced to lactic acid during exertion. Lactic acid is reoxidized and partially retransformed to glycogen during rest. Pyruvate can also act as an antioxidant to neutralize oxygen radicals in the cell and can be used in the multifunction oxidase system to reverse cytotoxicity.

The pyruvate in the present invention may be selected from the group consisting of pyruvic acid, pharmaceutically acceptable salts of pyruvic acid, and mixtures thereof. In general, the pharmaceutically acceptable salts of pyruvic acid may be alkali salts and alkaline earth salts. Preferably, the pyruvate is selected from the group consisting of pyruvic acid, sodium pyruvate, potassium pyruvate, magnesium pyruvate,

calcium pyruvate, zinc pyruvate, manganese pyruvate, and mixtures thereof. More preferably, the pyruvate is selected from the group of salts consisting of sodium pyruvate, potassium pyruvate, magnesium pyruvate, calcium pyruvate, zinc pyruvate, manganese pyruvate, and mixtures thereof. Most preferably, the pyruvate is sodium pyruvate.

The amount of pyruvate present in the cytoprotective compositions of the present invention is a therapeutically effective amount. A therapeutically effective amount of pyruvate is that amount of pyruvate necessary to increase the proliferation and resuscitation rate of mammalian cells. The exact amount of pyruvate is a matter of preference subject to such factors as the type of condition being treated as well as the other ingredients in the composition. When the cytoprotective composition contains two components, pyruvate is preferably present in the cytoprotective composition in an amount from about 10% to about 75%, preferably from about 20% to about 60%, and more preferably from about 25% to about 55%, by weight of the cytoprotective composition. When the cytoprotective composition contains three components, pyruvate is preferably present in the cytoprotective composition in an amount from about 10% to about 50%, preferably from about 20% to about 45%, and more preferably from about 25% to about 40%, by weight of the cytoprotective composition.

L-Lactic acid ((S)-2-hydroxypropanoic acid, (+) *alpha*-hydroxypropionic acid, $CH_3CHOHCOOH$) or lactate occurs in small quantities in the blood and muscle fluid of mammals. Lactic acid concentration increases in muscle and blood after vigorous activity. Lactate is a component in the cellular feedback mechanism and inhibits the natural respiratory bursting process of cells thereby suppressing the production of oxygen radicals.

The lactate in the present invention may be selected from the group consisting of lactic acid, pharmaceutically acceptable salts of lactic acid, and mixtures thereof. In general, the pharmaceutically acceptable salts of lactic acid may be alkali salts and alkaline earth salts. Preferably, the lactate is selected from the group consisting of lactic acid, sodium lactate, potassium lactate, magnesium lactate, calcium lactate, zinc lactate, manganese lactate, and mixtures thereof. More preferably, the lactate is selected from the group consisting of lactic acid, sodium lactate, potassium lactate, magnesium lactate, calcium lactate, zinc lactate, manganese lactate, and mixtures thereof. Most preferably, the lactate is lactic acid.

The amount of lactate present in the cytoprotective compositions of the present invention is a therapeutically effective amount. A therapeutically effective amount of lactate is that amount of lactate necessary to increase the proliferation and resuscitation rate of mammalian cells. For a composition, a therapeutically effective amount of lactate is that amount necessary to suppress the respiratory bursting process of white blood cells to protect and resuscitate the mammalian cells. In general, a therapeutically effective amount of lactate in a composition is from about 5 to about 10 times the amount of lactate normally found in serum. The exact amount of lactate is a matter of preference subject to such factors as the type of condition being treated as well as the other ingredients in the composition. In a preferred embodiment, lactate is present in the cytoprotective composition in an amount from about 10% to about 50%, preferably from about 20% to about 45%, and more preferably from about 25% to about 40%, by weight of the cytoprotective composition.

Antioxidants are substances which inhibit oxidation or suppress reactions promoted by oxygen or peroxides. Antioxidants, especially lipid-soluble

antioxidants, can be absorbed into the cellular membrane to neutralize oxygen radicals and thereby protect the membrane. The antioxidants useful in the present invention may be selected from the group consisting of Vitamin A (retinol), Vitamin A_2 (3, 4-didehydroretinol), all forms of carotene such as *alpha*-carotene, *beta*-carotene (*beta*, *beta*-carotene), *gamma*-carotene, *delta*-carotene, Vitamin C (ascorbic acid, L-ascorbic acid), all forms of tocopherol such as Vitamin E (*alpha*-tocopherol, 3,4-dihydro-2,5,7,8-tetramethyl-2-(4,8,12-trimethyltridecyl)-2H-1-benzopyran-6-ol), *beta*-tocopherol, *gamma*-tocopherol, and *delta*-tocopherol, and mixtures thereof. Preferably, the antioxidant is selected from the group of lipid-soluble antioxidants consisting of Vitamin A, *beta*-carotene, Vitamin E, and mixtures thereof. More preferably, the antioxidant is Vitamin E.

The amount of antioxidant present in the cytoprotective compositions of the present invention is a therapeutically effective amount. A therapeutically effective amount of antioxidant is that amount of antioxidant necessary to increase the proliferation and resuscitation rate of mammalian cells. The exact amount of antioxidant is a matter of preference subject to such factors as the type of condition being treated as well as the other ingredients in the composition. When the cytoprotective composition contains two components, the antioxidant is preferably present in the cytoprotective composition in an amount from about 10% to about 75%, preferably from about 20% to about 60%, and more preferably from about 25% to about 55%, by weight of the cytoprotective composition. When the cytoprotective composition contains three components, the antioxidant is preferably is present in the cytoprotective composition in an amount from about 10% to about 50%, preferably from about 20% to about 45%, and more preferably from about 25% to about 40%, by weight of the cytoprotective composition.

The mixture of saturated and unsaturated fatty acids in the present invention are those fatty acids required for the repair of mammalian cellular membranes and the production of new cells. Hence, the fatty acids are readily incorporated into the cell and are immediately available for the repair of injured cells and the proliferation of new cells. By providing the cell with the unsaturated fatty acids needed for repair, the need of the cell for unsaturated fatty acids is reduced and the need for high oxygen levels is also reduced. Accordingly, the presence of the mixtures of saturated and unsaturated fatty acids in the cytoprotective compositions significantly enhances the ability of pyruvate, lactate, and the antioxidant to inhibit reactive oxygen production.

Fatty acids are carboxylic acid compounds found in animal and vegetable fat and oil. Fatty acids are classified as lipids and are composed of chains of alkyl groups containing from 4 to 22 carbon atoms and 0-3 double bonds and characterized by a terminal carboxyl group, -COOH. Fatty acids may be saturated or unsaturated and may be solid, semisolid, or liquid. The most common saturated fatty acids are butyric acid (C_4), lauric acid (C_{12}), palmitic acid (C_{16}), and stearic acid (C_{18}). Unsaturated fatty acids are usually derived from vegetables and consist of alkyl chains containing from 16 to 22 carbon atoms and 0-3 double bonds with the characteristic terminal carboxyl group. The most common unsaturated fatty acids are oleic acid, linoleic acid, and linolenic acid (all C_{18} acids).

In general, the mixture of saturated and unsaturated fatty acids required for the repair of mammalian cellular membranes in the present invention may be derived from animal fats and waxes. Cells produce the chemical components and the energy required for cellular viability and store excess energy in the form of fat. Fat is adipose tissue stored between organs of the body

to furnish a reserve supply of energy. The preferred animal fats and waxes have a fatty acid composition similar to that of human fat and the fat contained in human breast milk. The preferred animal fats and waxes may be selected from the group consisting of human fat, chicken fat, cow fat (defined herein as a bovine domestic animal regardless of sex or age), sheep fat, horse fat, pig fat, and whale fat. The more preferred animal fats and waxes may be selected from the group consisting of human fat and chicken fat. The most preferred animal fat is human fat. Mixtures of other fats and waxes, such as vegetable waxes, marine oils (especially shark liver oil), and synthetic waxes and oils, which have a fatty acid composition similar to that of animal fats and waxes, and preferably to that of human fats and waxes, may also be employed. The mixture of saturated and unsaturated fatty acids may also be derived from animal and vegetable fats and waxes, and mixtures thereof.

In a preferred embodiment, the mixture of saturated and unsaturated fatty acids has a composition similar to that of human fat and comprises the following fatty acids: butyric acid, caproic acid, caprylic acid, capric acid, lauric acid, myristic acid, myristoleic acid, palmitic acid, palmitoleic acid, stearic, oleic acid, linoleic acid, linolenic acid, arachidic acid, and gaddoleic acid. Preferably, butyric acid, caproic acid, caprylic acid, capric acid, lauric acid, myristic acid, myristoleic acid, palmitic acid, palmitoleic acid, stearic, oleic acid, linoleic acid, linolenic acid, arachidic acid, and gaddoleic acid are present in the mixture in about the following percentages by weight, respectively (carbon chain number and number of unsaturations are shown parenthetically, respectively): 0.2%-0.4% (C_4), 0.1% (C_6), 0.3%-0.8% (C_8), 2.2%-3.5% (C_{10}), 0.9%-5.5% (C_{12}), 2.8%-8.5% (C_{14}), 0.1%-0.6% ($C_{14:1}$), 23.2%-24.6% (C_{16}), 1.8%-3.0% ($C_{16:1}$), 6.9%-9.9% (C_{18}), 36.0%-36.5% ($C_{18:1}$), 20%-20.6% ($C_{18:2}$), 7.5-7.8% ($C_{18:3}$), 1.1%-4.9% (C_{20}), and 3.3%-6.4% ($C_{20:1}$).

In another preferred embodiment, the mixture of saturated and unsaturated fatty acids is typically chicken fat comprising the following fatty acids: lauric acid, myristic acid, myristoleic acid, pentadecanoic acid, palmitic acid, palmitoleic acid, margaric acid, margaroleic acid, stearic, oleic acid, linoleic acid, linolenic acid, arachidic acid, and gaddoleic acid. Preferably, lauric acid, myristic acid, myristoleic acid, pentadecanoic acid, palmitic acid, palmitoleic acid, margaric acid, margaroleic acid, stearic, oleic acid, linoleic acid, linolenic acid, arachidic acid, and gaddoleic acid are present in the mixture in about the following percentages by weight, respectively: 0.1% (C_{12}), 0.8% (C_{14}), 0.2% ($C_{14:1}$), 0.1% (C_{15}), 25.3% (C_{16}), 7.2% ($C_{16:1}$), 0.1% (C_{17}), 0.1% ($C_{17:1}$), 6.5% (C_{18}), 37.7% ($C_{18:1}$), 20.6% ($C_{18:2}$), 0.8% ($C_{18:3}$), 0.2% (C_{20}), and 0.3% ($C_{20:1}$), all percentages +/- 10%.

The above fatty acids and percentages thereof present in the fatty acid mixture are given as an example. The exact type of fatty acid present in the fatty acid mixture and the exact amount of fatty acid employed in the fatty acid mixture may be varied in order to obtain the result desired in the final product and such variations are now within the capabilities of those skilled in the art without the need for undue experimentation.

The amount of fatty acids present in the cytoprotective compositions of the present invention is a therapeutically effective amount. A therapeutically effective amount of fatty acids is that amount of fatty acids necessary to increase the repair of cellular membranes and resuscitation rate of mammalian cells. The exact amount of fatty acids employed is subject to such factors as the type and distribution of fatty acids employed in the mixture, the type of condition being treated, and the other ingredients in the composition.

When the cytoprotective composition contains two components, the fatty acids are preferably present in the cytoprotective composition in an amount from about 10% to about 75%, preferably from about 20% to about 60%, and more preferably from about 25% to about 55%, by weight of the cytoprotective composition. When the cytoprotective composition contains three components, the fatty acids are preferably present in the cytoprotective composition in an amount from about 10% to about 50%, preferably from about 20% to about 45%, and more preferably from about 25% to about 40%, by weight of the cytoprotective composition.

In accord with the present invention, the therapeutic cytoprotective compositions for preventing and reducing injury to mammalian cells from a medicament having cytotoxic properties may be selected from the group consisting of:

(1) (a) pyruvate selected from the group consisting of pyruvic acid, pharmaceutically acceptable salts of pyruvic acid, and mixtures thereof;

(b) an antioxidant; and

(c) a mixture of saturated and unsaturated fatty acids wherein the fatty acids are those fatty acids required for the repair of cellular membranes and resuscitation of mammalian cells;

(2) (a) pyruvate selected from the group consisting of pyruvic acid, pharmaceutically acceptable salts of pyruvic acid, and mixtures thereof;

(b) lactate selected from the group consisting of lactic acid, pharmaceutically acceptable salts of lactic acid, and mixtures thereof; and

(c) a mixture of saturated and unsaturated fatty acids wherein the fatty acids are those fatty acids required for the repair of cellular membranes and resuscitation of mammalian cells;

(3) (a) an antioxidant; and

(b) a mixture of saturated and unsaturated fatty acids wherein the fatty acids are those fatty acids

required for the repair of cellular membranes and resuscitation of mammalian cells;

(4) (a) lactate selected from the group consisting of lactic acid, pharmaceutically acceptable salts of lactic acid, and mixtures thereof;

(b) an antioxidant; and

(c) a mixture of saturated and unsaturated fatty acids wherein the fatty acids are those fatty acids required for the repair of cellular membranes and resuscitation of mammalian cells; and

(5) (a) pyruvate selected from the group consisting of pyruvic acid, pharmaceutically acceptable salts of pyruvic acid, and mixtures thereof; and

(b) an antioxidant.

In a preferred embodiment, the cytoprotective compositions for treating mammalian cells, preferably epidermal keratinocytes, may be selected from the group consisting of:

(1) (a) pyruvate selected from the group consisting of pyruvic acid, pharmaceutically acceptable salts of pyruvic acid, and mixtures thereof;

(b) an antioxidant; and

(c) a mixture of saturated and unsaturated fatty acids wherein the fatty acids are those fatty acids required for the repair of cellular membranes and resuscitation of mammalian cells;

(2) (a) pyruvate selected from the group consisting of pyruvic acid, pharmaceutically acceptable salts of pyruvic acid, and mixtures thereof;

(b) lactate selected from the group consisting of lactic acid, pharmaceutically acceptable salts of lactic acid, and mixtures thereof; and

(c) a mixture of saturated and unsaturated fatty acids wherein the fatty acids are those fatty acids required for the repair of cellular membranes and resuscitation of mammalian cells; and

(3) (a) an antioxidant; and

(b) a mixture of saturated and unsaturated fatty acids wherein the fatty acids are those fatty acids required for the repair of cellular membranes and resuscitation of mammalian cells; and

(5) (a) pyruvate selected from the group consisting of pyruvic acid, pharmaceutically acceptable salts of pyruvic acid, and mixtures thereof; and

(b) an antioxidant.

In a more preferred embodiment, the cytoprotective compositions for treating mammalian cells, preferably epidermal keratinocytes, may be selected from the group consisting of:

(1) (a) pyruvate selected from the group consisting of pyruvic acid, pharmaceutically acceptable salts of pyruvic acid, and mixtures thereof;

(b) an antioxidant; and

(c) a mixture of saturated and unsaturated fatty acids wherein the fatty acids are those fatty acids required for the repair of cellular membranes and resuscitation of mammalian cells; and

(5) (a) pyruvate selected from the group consisting of pyruvic acid, pharmaceutically acceptable salts of pyruvic acid, and mixtures thereof; and

(b) an antioxidant.

In a most preferred embodiment, the cytoprotective compositions for treating mammalian cells, preferably epidermal keratinocytes, comprise:

(a) pyruvate selected from the group consisting of pyruvic acid, pharmaceutically acceptable salts of pyruvic acid, and mixtures thereof;

(b) an antioxidant; and

(c) a mixture of saturated and unsaturated fatty acids wherein the fatty acids are those fatty acids required for the repair of cellular membranes and resuscitation of mammalian cells.

The present invention extends to methods for making the therapeutic cytoprotective compositions. In general, a cytoprotective composition is made by forming an admixture of the components of the composition. In a first embodiment, a cytoprotective composition is made by forming an admixture of (a) a pyruvate, (b) an antioxidant, and (c) a mixture of saturated and unsaturated fatty acids. In a second embodiment, a cytoprotective composition is made by forming an admixture of (a) a pyruvate, (b) a lactate, and (c) a mixture of saturated and unsaturated fatty acids. In a third embodiment, a cytoprotective composition is made by forming an admixture of (a) an antioxidant, and (b) a mixture of saturated and unsaturated fatty acids. In a fourth embodiment, a cytoprotective composition is made by forming an admixture of (a) a lactate, (b) an antioxidant, and (c) a mixture of saturated and unsaturated fatty acids. In a fifth embodiment, a cytoprotective composition is made by forming an admixture of (a) a pyruvate, and (b) an antioxidant.

For some applications, the admixture may be formed in a solvent such as water. If necessary, the pH of the solvent is adjusted to a range from about 3.5 to about 8.0, and preferably from about 4.5 to about 7.5, and more preferably about 6.0 to about 7.4. The admixture is then sterile filtered. Other ingredients may also be incorporated into the cytoprotective composition as dictated by the nature of the desired composition as well known by those having ordinary skill in the art. The ultimate cytoprotective compositions are readily prepared using methods generally known in the pharmaceutical arts.

Once prepared, the inventive therapeutic cytoprotective compositions may be stored for future use or may be formulated in effective amounts with a cytotoxic agent to form cytoprotective pharmaceutical compositions. The combination of the cytoprotective

compositions of the present invention and the medicament cytotoxic to cells provides a cytoprotective pharmaceutical composition having the ability to prevent and reduce injury to mammalian cells from the cytotoxic medicament and increase the resuscitation rate of injured mammalian cells. The dose level of the cytotoxic medicament in the cytoprotective pharmaceutical composition may thereby be raised to higher than normal levels.

The cytotoxic agents which may be used in the cytoprotective pharmaceutical compositions of the present invention may be selected from a wide variety of medicaments. For example, medicaments taken on a long term regimen tend to cause liver, kidney, tissue, and other toxicity problems. In addition, certain cytotoxic medicaments, such as potent chemotherapeutic medicaments used to treat malignant tissues, are believed to stimulate release of significant amounts of reactive oxygen species by mammalian tissues which can cause oxidative injury. Combination of the cytoprotective compositions of the present invention with such cytotoxic medicaments may inhibit induction of reactive oxygen production while simultaneously decreasing side effects of such medicaments. By decreasing the side effects of such medicaments, the dosage levels of the medicaments may be increased thereby increasing the therapeutic effect of the medicaments. For example, the cytoprotective compositions may be used in topical cytoprotective pharmaceutical compositions in combination with cytotoxic medicaments such as epithelial cell cohesiveness reducers such as tretinoin (Retin A), dermatological abradants, and anti-inflammatories, to protect and enhance the resuscitation rate of the injured mammalian cells. The cytoprotective compositions may also be used in ingestible cytoprotective pharmaceutical compositions in combination with medicaments that cause cytotoxic side effects such as anti-tumor, anti-viral, and antibacterial medicaments including the lipid

regulating agents gemfibrozil and lovastatin, centrally acting anticholinesterases such as tacrine, chemotherapeutic medicaments such as the anthracycline antibiotic doxorubicin, gastric irritants such as acetylsalicylic acid and ibuprofen, to protect and enhance the resuscitation rate of the injured mammalian cells.

In one preferred embodiment, the medicament having cytotoxic properties in the cytoprotective pharmaceutical compositions is selected from the group consisting of doxorubicin, gemfibrozil, lovastatin, and tacrine. In a more preferred embodiment, the medicament having cytotoxic properties in the cytoprotective pharmaceutical compositions is selected from the group consisting of doxorubicin, gemfibrozil, and tacrine. In a most preferred embodiment, the medicament having cytotoxic properties is doxorubicin. Doxorubicin (Adriamycin) is a cytotoxic anthracycline antibiotic reported to produce regression in disseminated neoplastic conditions such as in various leukemias, tumors, neuroblastomas, sarcomas, and carcinomas. Gemfibrozil (Lopid) is a lipid regulating agent which lowers elevated serum lipids primarily by decreasing serum triglyceride with a variable reduction in total serum cholesterol. Lovastatin (Mevacor) is a cholesterol lowering agent which inhibits the enzymatic biosynthesis of cholesterol. Tacrine (Cognex, 1,2,3,4-tetrahydro-9-acridinamine) is a centrally active anticholinesterase useful as a cognition activator. Tacrine has undergone clinical trials for use in treating severe Alzheimer's disease (presenile dementia).

In another preferred embodiment, the medicament having cytotoxic properties in the cytoprotective pharmaceutical compositions is an anticancer agent. Nonlimiting examples of anticancer agents include chemically reactive drugs having nonspecific action, anti-metabolites, antibiotics, plant products, hormones,

and other miscellaneous chemotherapeutic agents. Chemically reactive drugs having nonspecific action include alkylating agents and N-alkyl-N-nitroso compounds. Examples of alkylating agents include nitrogen mustards, azridines (ethylenimines), sulfonic acid esters, and epoxides. Anti-metabolites are compounds that interfere with the formation or utilization of a normal cellular metabolite and include amino acid antagonists, vitamin and coenzyme antagonists, and antagonists of metabolites involved in nucleic acid synthesis such as glutamine antagonists, folic acid antagonists, pyrimidine antagonists, and purine antagonists. Antibiotics are compounds produced by microorganisms that have the ability to inhibit the growth of other organisms and include actinomycins and related antibiotics, glutarimide antibiotics, sarkomycin, fumagillin, streptonigrin, tenuazonic acid, actinogan, peptinogan, and anthracyclic antibiotics such as doxorubicin. Plant products include colchicine, podophyllotoxin, and vinca alkaloids. Hormones include those steroids used in breast and prostate cancer and corticosteroids used in leukemias and lymphomas. Other miscellaneous chemotherapeutic agents include urethan, hydroxyurea, and related compounds; thiosemicarbazones and related compounds; phthalanilide and related compounds; and triazenes and hydrazines. In a preferred embodiment, the anticancer agent is an antibiotic. In a more preferred embodiment, the anticancer agent is doxorubicin. In a most preferred embodiment, the anticancer agent is doxorubicin.

In a specific embodiment, the invention is directed at a cytoprotective pharmaceutical composition for preventing and reducing injury to mammalian cells from a medicament having cytotoxic properties which comprises:

(A) a cytoprotective composition selected from the group consisting of:

(1) (a) pyruvate selected from the group consisting of pyruvic acid, pharmaceutically acceptable salts of pyruvic acid, and mixtures thereof;

(b) an antioxidant; and

(c) a mixture of saturated and unsaturated fatty acids wherein the fatty acids are those fatty acids required for the resuscitation of injured mammalian cells; and

(2) (a) pyruvate selected from the group consisting of pyruvic acid, pharmaceutically acceptable salts of pyruvic acid, and mixtures thereof; and

(b) an antioxidant; and

(B) a medicament having cytotoxic properties.

In another form of this embodiment, the cytoprotective compositions of the present invention, may be combined in an immediate release form with an anticancer medicament having cytotoxic properties in a timed release form to provide a timed release cytoprotective pharmaceutical composition. In this embodiment, the timed release composition releases the cytoprotective composition substantially immediately and releases the cytotoxic chemotherapeutic medicament after a suitable period of time, for example from one to 24 hours after releasing the cytoprotective composition, to selectively protect non-cancerous cells in the presence of cancerous cells against the cytotoxic chemotherapeutic medicament. Cancer cells, unlike normal cells or benign tumor cells, exhibit the properties of invasion and metastasis and are highly anaplastic. Because cancerous cells have a rapid metabolism, cancerous cells will rapidly consume the protective cytoprotective composition and will not be protected by the cytoprotective compositions when the chemotherapeutic medicament is subsequently released. Non-cancerous cells which do not have such a rapid metabolism will not rapidly consume the cytoprotective compositions and will be protected when the chemotherapeutic medicament is subsequently released.

In a specific embodiment, the invention is direct at a timed-release cytoprotective pharmaceutical composition for selectively protecting non-cancerous mammalian cells in the presence of cancerous mammalian cells from an anticancer medicament having cytotoxic properties which comprises:

(A) a cytoprotective composition in immediate release form selected from the group consisting of:

(1) (a) pyruvate selected from the group consisting of pyruvic acid, pharmaceutically acceptable salts of pyruvic acid, and mixtures thereof;

(b) an antioxidant; and

(c) a mixture of saturated and unsaturated fatty acids wherein the fatty acids are those fatty acids required for the resuscitation of injured mammalian cells; and

(2) (a) pyruvate selected from the group consisting of pyruvic acid, pharmaceutically acceptable salts of pyruvic acid, and mixtures thereof; and

(b) an antioxidant; and

(B) an anticancer medicament having cytotoxic properties in timed-release form;

wherein the cytoprotective composition is released substantially immediately and the anticancer medicament is released after a period of time sufficient such that the cancerous cells have substantially metabolized the cytoprotective composition and the non-cancerous cells have not substantially metabolized the cytoprotective composition.

A suitable or sufficient period of time is that period of time wherein the cancerous cells have substantially metabolized the cytoprotective composition and the non-cancerous cells have not substantially metabolized the cytoprotective composition. The period of time should not be so long that the non-cancerous cells substantially metabolize the cytoprotective composition and are unprotected. The exact time is

subject to such factors as the type and quantity of cytoprotective composition employed, the medicament having cytotoxic properties used, and the type of cancerous cells and non-cancerous cells being treated. Thus, the period of time may be varied in order to obtain the result desired and such variations are within the capabilities of those skilled in the art without the need for undue experimentation.

The present invention extends to methods for making the cytoprotective pharmaceutical composition. In general, a cytoprotective pharmaceutical composition is made by forming an admixture of the components of the composition. The cytoprotective compositions may be prepared using standard techniques and equipment known to those skilled in the art. The apparatus useful in accordance with the present invention comprises apparatus well known in the chemical and biochemical arts, and therefore the selection of the specific apparatus will be apparent to the artisan.

In one embodiment, a cytoprotective pharmaceutical composition is made by forming an admixture of the cytoprotective composition and the medicament having cytotoxic properties. In a second embodiment, a timed-release cytoprotective pharmaceutical composition is made by forming an admixture of the cytoprotective composition in immediate release form and the anticancer medicament having cytotoxic properties in timed-release form.

The present invention extends to methods for using the therapeutic cytoprotective compositions. In one embodiment, the cytoprotective compositions of the present invention may be administered to cells concurrently with a cytotoxic medicament. In another embodiment, the cytoprotective compositions of the present invention may be administered to cells prior to the administration of a cytotoxic anticancer medicament

to selectively protect non-cancerous cells in the presence of cancerous cells against the anticancer agent.

In a specific embodiment, the invention is directed at a method for protecting mammalian cells from a medicament having cytotoxic properties which comprises the steps of:

(A) providing a cytoprotective composition selected from the group consisting of:

(1) (a) pyruvate selected from the group consisting of pyruvic acid, pharmaceutically acceptable salts of pyruvic acid, and mixtures thereof;

(b) an antioxidant; and

(c) a mixture of saturated and unsaturated fatty acids wherein the fatty acids are those fatty acids required for the resuscitation of injured mammalian cells; and

(2) (a) pyruvate selected from the group consisting of pyruvic acid, pharmaceutically acceptable salts of pyruvic acid, and mixtures thereof; and

(b) an antioxidant; and

(B) providing an anticancer medicament having cytotoxic properties; and

(C) administering the cytoprotective composition from step (A) and the medicament from step (B) concurrently to mammalian cells to protect the mammalian cells from the medicament having cytotoxic properties.

In another specific embodiment, the invention is direct at a method for selectively protecting non-cancerous mammalian cells in the presence of cancerous mammalian cells from an anticancer medicament having cytotoxic properties which comprises the steps of:

(A) providing a cytoprotective composition in an immediate release form selected from the group consisting of:

(1) (a) pyruvate selected from the group consisting of pyruvic acid, pharmaceutically acceptable salts of pyruvic acid, and mixtures thereof;

(b) an antioxidant; and

(c) a mixture of saturated and unsaturated fatty acids wherein the fatty acids are those fatty acids required for the resuscitation of injured mammalian cells; and

(2) (a) pyruvate selected from the group consisting of pyruvic acid, pharmaceutically acceptable salts of pyruvic acid, and mixtures thereof; and

(b) an antioxidant; and

(B) providing an anticancer medicament having cytotoxic properties in timed-release form; and

(C) administering the cytoprotective composition from step (A) and the medicament from step (B) concurrently to mammalian cells to selectively protect non-cancerous mammalian cells in the presence of cancerous mammalian cells from the anticancer medicament having cytotoxic properties;

wherein the cytoprotective composition is released substantially immediately and the anticancer medicament is released after a period of time sufficient such that the cancerous cells have substantially metabolized the cytoprotective composition and the non-cancerous cells have not substantially metabolized the cytoprotective composition.

In yet another specific embodiment, the invention is direct at a method for selectively protecting non-cancerous mammalian cells in the presence of cancerous mammalian cells from an anticancer medicament having cytotoxic properties which comprises the steps of:

(A) administering to mammalian cells a cytoprotective composition to prevent and reduce injury to the mammalian cells selected from the group consisting of:

(1) (a) pyruvate selected from the group consisting of pyruvic acid, pharmaceutically acceptable salts of pyruvic acid, and mixtures thereof;

(b) an antioxidant; and

(c) a mixture of saturated and unsaturated fatty acids wherein the fatty acids are those fatty acids required for the resuscitation of injured mammalian cells; and

(2) (a) pyruvate selected from the group consisting of pyruvic acid, pharmaceutically acceptable salts of pyruvic acid, and mixtures thereof; and

(b) an antioxidant; and

(B) waiting a period of time sufficient such that the cancerous cells have substantially metabolized the cytoprotective composition and the non-cancerous cells have not substantially metabolized the cytoprotective composition; and

(C) administering the cytotoxic anticancer medicament to the mammalian cells to treat the cancerous cells which are unprotected by the cytoprotective composition and the non-cancerous cells which are protected by the cytoprotective composition to thereby increase the therapeutic effect of the anticancer medicament.

Methods for administering the cytoprotective compositions of the present invention to mammalian cells will vary depending upon the particular condition being treated and the cytotoxic agent employed. In general, the cytoprotective compositions will be administered in the same manner as the cytotoxic agent. Of course, the type of carrier will vary depending upon the mode of administration desired for the pharmaceutical composition as is conventional in the art.

The cytoprotective compositions of the present invention may be administered parenterally, in the form of sterile solutions or suspensions, such as intravenously, intramuscularly, or subcutaneously. The cytoprotective compositions may also be administered topically. Non-oral topical compositions employ non-oral topical vehicles, such as oils, petrolatum bases, emulsions, lotions, creams, gel formulations, foams,

ointments, sprays, salves, and films, which are intended to be applied to the skin or body cavity and are not intended to be taken by mouth. Oral topical compositions employ oral vehicles, such as mouthwashes, rinses, oral sprays, suspensions, bioadhesives, and dental gels, which are intended to be taken by mouth but are not intended to be ingested. The cytoprotective compositions may also be administered orally, in the form of pills, tablets, capsules, troches, and the like, as well as sublingually, rectally, or transcutaneously with a suitable pharmaceutically acceptable carrier for that particular mode of administration as is conventional in the art.

It is especially advantageous to formulate the pharmaceutical compositions in dosage unit forms for ease of administration and uniformity of dosage. The term dosage unit forms as used herein refers to physically discrete units suitable for use as a unitary dosage, each unit containing a predetermined quantity of active ingredient calculated to produce the desired therapeutic effect in association with the pharmaceutical carrier.

For parental therapeutic administration, the cytoprotective compositions of the present invention may be incorporated into a sterile solution or suspension. These preparations should contain at least about 0.1% of the inventive composition, by weight, but this amount may be varied to between about 0.1% and about 50% of the inventive composition, by weight of the parental composition. The exact amount of the inventive composition present in such compositions is such that a suitable dosage level will be obtained. Preferred compositions and preparations according to the present invention are prepared so that a paranteral dosage unit contains from between about 0.5 milligrams to about 100 milligrams of the inventive composition.

Suitable carriers include propylene glycol-alcohol-water, isotonic water, sterile water for

injection (USP), emulphor^{TM}-alcohol-water, cremophor-EL^{TM} or other suitable carriers known to those skilled in the art. The sterile solutions or suspensions may also include the following adjuvants: a sterile diluent, such as water for injection, saline solution, fixed oils, polyethylene glycol, glycerine, propylene glycol, or other synthetic solvent; antibacterial agents, such as benzyl alcohol or methyl paraben; antioxidants, such as ascorbic acid or sodium metabisulfite; chelating agents, such as ethylenediaminetetraacetic acid (EDTA); buffers, such as acetates, citrates or phosphates; and agents for the adjustment of tonicity, such as sodium chloride or dextrose. The parental preparations may be enclosed in ampules, disposable syringes, or multiple dose vials made of glass or plastic.

In another form of the invention, the therapeutic cytoprotective composition is incorporated into a non-oral topical vehicle which may be in the form of oils, petrolatum bases, emulsions, lotions, creams, gels formulations, foams, ointments, sprays, salves, and films, and the like. Non-oral topical vehicles include water and pharmaceutically acceptable water-miscible organic solvents such as ethyl alcohol, isopropyl alcohol, propylene glycol, glycerin, and the like, and mixtures of these solvents. Typical non-toxic non-oral topical vehicles known in the pharmaceutical arts may be used. The non-oral topical cytoprotective compositions may also contain conventional additives employed in those products. Conventional additives include humectants, emollients, lubricants, stabilizers, dyes, and perfumes, providing the additives do not interfere with the therapeutic properties of the cytoprotective composition.

In another form of the invention, the cytoprotective composition is incorporated into an oral topical vehicle which may be in the form of a mouthwash, rinse, oral spray, suspension, dental gel, bioadhesive, and the like. Typical non-toxic oral vehicles known in

the pharmaceutical arts may be used in the present invention. The preferred oral vehicles are water, ethanol, and water-ethanol mixtures. The water-ethanol mixtures are generally employed in a weight ratio from about 1:1 to about 20:1, preferably from about 3:1 to about 20:1, and most preferably from about 3:1 to about 10:1, respectively. The pH value of the oral vehicle is generally from about 4 to about 7, and preferably from about 5 to about 6.5. An oral topical vehicle having a pH value below about 4 is generally irritating to the oral cavity and an oral vehicle having a pH value greater than about 7 generally results in an unpleasant mouth feel. The oral topical cytoprotective compositions may also contain conventional additives normally employed in those products. Conventional additives include a fluorine providing compound, a sweetening agent, a flavoring agent, a coloring agent, a humectant, a buffer, and an emulsifier, providing the additives do not interfere with the therapeutic properties of the cytoprotective composition.

In accordance with this invention, therapeutically effective amounts of the cytoprotective compositions of the present invention may be admixed with a topical vehicle to form a topical cytoprotective composition. These amounts are readily determined by those skilled in the art without the need for undue experimentation. In a preferred embodiment, the topical cytoprotective compositions will comprise the cytoprotective composition in an amount from about 0.1% to about 10% and a topical vehicle in a quantity sufficient to bring the total amount of composition to 100%, by weight of the topical cytoprotective composition. In a more preferred embodiment, the topical cytoprotective compositions will comprise the cytoprotective composition in an amount from about 0.1% to about 10%, and in a most preferred embodiment, the topical cytoprotective compositions will comprise the cytoprotective composition in an amount from about 0.1%

to about 8%, and a topical vehicle in a quantity sufficient to bring the total amount of composition to 100%, by weight of the topical cytoprotective composition.

The present invention extends to methods for preparing the topical cytoprotective compositions. In such a method, the topical cytoprotective composition is prepared by admixing a therapeutically effective amount of the cytoprotective composition of the present invention, the cytotoxic agent, and a topical vehicle. The final compositions are readily prepared using standard methods and apparatus generally known by those skilled in the pharmaceutical arts. The apparatus useful in accordance with the present invention comprises mixing apparatus well known in the pharmaceutical arts, and therefore the selection of the specific apparatus will be apparent to the artisan.

In a specific embodiment, the invention is directed at a cytoprotective pharmaceutical composition which comprises a pharmaceutically acceptable carrier and a therapeutically effective amount of a cytoprotective composition for preventing and reducing injury to mammalian cells from a medicament having cytotoxic properties, wherein the cytoprotective composition is selected from the group consisting of:

(1) (a) pyruvate selected from the group consisting of pyruvic acid, pharmaceutically acceptable salts of pyruvic acid, and mixtures thereof;

(b) an antioxidant; and

(c) a mixture of saturated and unsaturated fatty acids wherein the fatty acids are those fatty acids required for the resuscitation of injured mammalian cells; and

(2) (a) pyruvate selected from the group consisting of pyruvic acid, pharmaceutically acceptable salts of pyruvic acid, and mixtures thereof; and

(b) an antioxidant.

In another specific embodiment, the invention is directed at a method for preparing a cytoprotective pharmaceutical composition for protecting mammalian cells from a medicament having cytotoxic properties which comprises the steps of:

(A) providing a therapeutically effective amount of a cytoprotective composition which comprises:

(1) (a) pyruvate selected from the group consisting of pyruvic acid, pharmaceutically acceptable salts of pyruvic acid, and mixtures thereof;

(b) an antioxidant; and

(c) a mixture of saturated and unsaturated fatty acids wherein the fatty acids are those fatty acids required for the resuscitation of injured mammalian cells; and

(2) (a) pyruvate selected from the group consisting of pyruvic acid, pharmaceutically acceptable salts of pyruvic acid, and mixtures thereof; and

(b) an antioxidant; and

(B) providing a pharmaceutically acceptable carrier; and

(C) admixing the cytoprotective composition from step (A) and the pharmaceutically acceptable carrier from step (B) to form a pharmaceutical composition.

Throughout this application, various publications have been referenced. The disclosures in these publications are incorporated herein by reference in order to more fully describe the state of the art.

The present invention is further illustrated by the following examples which are not intended to limit the effective scope of the claims. All parts and percentages in the examples and throughout the specification and claims are by weight of the final composition unless otherwise specified.

EXAMPLES

These examples demonstrate the cytoprotective abilities of the therapeutic cytoprotective compositions of the present invention.

Methods

Isolation of Peripheral Blood Monocytes

Peripheral blood was obtained from a normal healthy volunteer by venipuncture using an EDTA-containing Vacutainer (Becton Dickinson Mountain View, Ca.). A total of 10 ml of peripheral blood was mixed in a ratio of 1:1 with Dulbecco's Minimal Essential Medium (DMEM, Grand Island Biologicals, GIBCO, Grand Island, N.Y.). The mixture was divided into 2 ml portions and each portion was layered onto 6 ml of Ficoll-Hypaque gradient mixture (Pharmacy, Inc., Piscataway, N.J.) and centrifuged in a Beckman T-J6 refrigerated centrifuge for 30 minutes at 1500 rpm and 4° C. After the cells were washed twice with phosphate buffered saline, the cells were resuspended in Hank's Balanced Salt Solution without Ca^{++}/Mg^{++} (GIBCO).

Culture of U937 and Peripheral Blood Monocytes

Peripheral blood monocytes and U937 monocytic leukemia tumor cells were placed in sterile culture flasks and maintained in culture using Dulbecco's Minimal Essential Medium, with 10% fetal calf serum, supplemented with 2mM glutamine and Pen/Strep. The cytotoxicity of the cytotoxic agent on the cells was analyzed by propidium iodide exclusion techniques and flow cytometric quantitation. Viability of the cells was quantified as the number of cells that excluded the vital dye trypan blue.

Preparation of Chemicals

Sodium pyruvate was dissolved in distilled water and the solution was adjusted to pH 7.4 with 1N sodium hydroxide solution. Solutions were sterile filtered. Stock solutions were prepared so that the vehicle would not be more than 1% of the total volume of the culture media.

A mixture of fatty acids derived from chicken fat was prepared by mixing 0.1% chicken fat with mineral oil to form an emulsified solution. Tween 80 was added to separate cultures of cells at similar concentrations and to examine possible vehicle effects.

Alpha-tocopherol phosphate (Sigma Chemical Company, St. Louis, MO) was added directly to the culture medium.

^{3}H-Thymidine Radiosotopic Incorporation Measurement of Cytotoxity

Cells were plated into 96 well dishes at a concentration of 10^{6} cells/well. Tritiated thymidine (1 uCi/well) was added and the cells were incubated for 4 hours at which time the cells were harvested using a Cambridge cell harvestor. The samples were then placed in scintillation vials containing scintillation fluid and counted. These studies yielded a measure of the ability of the cells to proliferate, which is a measure of viability.

The results from the tritiated thymidine incorporation assay, a measure of DNA synthesis and cellular proliferation, correlated directly with the results from the dye exclusion viability assay. Because the tritiated thymidine incorporation assay is a more quantitative assay, the tritiated thymidine incorporation assay was used for the remainder of the studies.

A dose response curve for Doxorubicin (Adriamycin) alone was constructed. Doxorubicin is an anthracycline antibiotic used as a first line agent in a number of neoplastic conditions and is a well characterized cytotoxic agent. Doses and times examined ranged from 0.1, 0.5, 1, 5, 10, 25, and 50 ug/ml of Doxorubicin for 20-60 minutes and 24 hours. The range of optimal concentrations for cytotoxity of Doxorubicin was established for u937 monocytic tumor cells to be 0.5, 1 and 5 ug at 24 hours and 10 ug at 1-2 hours, see Figures 1 and 2.

The cytoprotective agents (sodium pyruvate, Vitamin E, and fatty acids) alone, and in combination, were examined for their ability to decrease the cytotoxity of Doxorubicin to U937 monocytic leukemia cells and normal peripheral blood monocytes. Optimal concentrations of the single ingredients of sodium pyruvate, Vitamin E, and fatty acids were examined. The optimal concentrations of the agents that were able to protect cells against Doxorubicin induced cytotoxity were as follows: 10-50 U Vitamin E, 0.5% fatty acids, and 5 mM of sodium pyruvate, see Figure 3.

Window of susceptibility studies were conducted to determine the optimal treatment time of the cells with the cytoprotective agents prior to treatment of the cells with the cytotoxic agent. The normal cells and U937 leukemic tumor cells were pretreated separately in "wash out" studies with the single agents alone, and in combination, at the optimal concentration described above for various time periods, washed with fresh medium to remove the agents, and treated with the cytotoxic agent. The co-culture of normal and U937 leukemic tumor cells was treated essentially in the same manner except that the cells were not treated separately, but co-cultured. The optimal pretreatment time of the cells with the cytoprotective agents was found to be 24 hours prior to

treatment of the cells with Doxorubicin. The cells were then placed in culture medium without the protective agents. The length of time that the cytoprotection lasted was 24 hours following Doxorubicin treatment. At this time, peripheral cell viability is a limiting factor because these cells are normal cells and do not remain in culture for extended periods of time.

Normal and U937 tumor cells were co-cultured and the cytotoxity of Doxorubicin on the cells was determined by viability assays which examined the differential ability of the cytoprotective compositions alone, and in combinations, to protect the normal cells from the cytotoxity of the chemotherapeutic agent.

The cells were isolated and examined for morphological evidence of cytotoxicity or prevention of cytotoxicity. These studies determined the cytoprotective effect of the single agents and the combination of agents on the normal and tumor cells. DNA synthesis studies using ^{3}H-thymidine (1 uCi/well) were carried out 4 hours prior to termination of the experiment to determine the effect of the formulations on the proliferation of the cells as a measure of the prevention of cytotoxicity and the extent of Doxorubicin-induced cytotoxicity. Propidium iodide exclusion analysis was carried out for direct quantitation of the cytotoxicity and the prevention of cytotoxicity. Each set of studies was performed in triplicate so that statistical analysis of the significant differences between the treatment groups could be conducted.

The effect of the cytoprotective agents on the co-culture of tumor and normal cells was very different from the effect of these agents on the individual cell types alone. An interaction between the normal cells and the tumor cells must cause the viability of the tumor cells to be significantly diminished. The cytoprotective combination of 5 mM sodium pyruvate, 0.5% fatty acids,

and 10 U Vitamin E provided significant protection to the normal peripheral monocytes and did not protect the tumor cells from the effects of the cytotoxic agent.

Wash-out studies were conducted to determine viability of the peripheral blood monocytes co-cultured with U937 monocytic leukemia cells after 24 hour pretreatment of the cells with the cytoprotective agents followed by administration of Doxorubicin. With no Doxorubicin treatment, the viability of the control normal peripheral cells was enhanced from 55% to 68% with the use of 5 mM sodium pyruvate and 0.5% fatty acids, see Figure 3. With no Doxorubicin treatment, the viability of the control U937 cells was enhanced from 43% to 62% with the use of the combination of the cytoprotective composition, 5 mM sodium pyruvate, 10 U Vitamin E, and 0.5% fatty acids, see Figure 3.

Pretreatment with a combination of 10 U Vitamin E and 5 mM sodium pyruvate prevented cytotoxity to normal peripheral blood monocytes with a concentration of 0.5 ug/ml Doxorubicin (53% to 68% viable), see Figure 9. Pretreatment with a combination of 5 mM sodium pyruvate, 10 U Vitamin E, and 0.5% fatty acids prevented cytotoxity to peripheral blood monocytes with a concentration of 1 ug/ml Doxorubicin (47% to 69% viable), see Figure 13. Pretreatment with the single agent 50 U Vitamin E prevented cytotoxity to U937 tumor cells induced by 1 ug/ml Doxorubicin (42% to 62% viable), see Figure 7.

The viability of cultured peripheral monocytes without Doxorubicin was 66% and increased to 75% with the cytoprotective combination of 5 mM sodium pyruvate, 10 U Vitamin E, and 0.5% fatty acids, see Figure 13. The viability of cultured peripheral monocytes treated with 0.5 ug/ml Doxorubicin was 47% and increased to 63.5% when pretreated with the cytoprotective combination of 5 mM sodium pyruvate, 10 U Vitamin E, and 0.5% fatty acids,

see Figure 13. The viability of cultured peripheral monocytes treated with 1 ug/ml Doxorubicin was 42% and increased to 66% when pretreated with the cytoprotective combination of 5 mM sodium pyruvate, 10 U Vitamin E, and 0.5% fatty acids, see Figure 13.

The viability of cultured U937 tumor cells without Doxorubicin was 67% and did not increase when treated with any of the agents, see Figure 13. The viability of cultured U937 tumor cells with 0.5 ug/ml Doxorubicin treatment was 47% and the highest increase in viability occurred with pretreatment of 50 U Vitamin E and 0.5% fatty acids, see Figure 12. The viability of cultured U937 tumor cells with 1 ug/ml Doxorubicin treatment was 45% and the highest increase in viability occurred with pretreatment of 10 U Vitamin E and 0.5% fatty acids, see Figure 12.

Optimal concentrations of the cytoprotective agents to prevent Doxorubicin-induced cytotoxity were found to be 5 mM sodium pyruvate, 10-50 U Vitamin E, and 0.5% fatty acids. In wash-out studies, the cytoprotective combination of sodium pyruvate, Vitamin E, and fatty acids and the combination of 5mM sodium pyruvate and 10 U Vitamin E protected the normal peripheral blood monocytes from Doxorubicin-induced cytotoxity, see Figure 13. Vitamin E alone and fatty acids alone prevented the cytotoxity of Doxorubicin in U937 cells, see Figure 11. When normal peripheral blood monocytes were co-cultured with U937 monocytic leukemia tumor cells, the cytoprotective combination of 5 mM sodium pyruvate, 0.5% fatty acids, and 10 U Vitamin E provided significant protection to the normal peripheral monocytes from Doxorubicin-induced cytotoxity and did not protect the tumor cells from the effects of the cytotoxic agent, see Figure 24.

These results show that the combination of agents 5 mM sodium pyruvate, 0.5% fatty acids, and 10 U

and 50U Vitamin E are useful as selective cytoprotective agents for use with compounds that are toxic to normal cells as well as tumor cells.

The invention being thus described, it will be obvious that the same may be varied in many ways. Such variations are not to be regarded as a departure from the spirit and scope of the invention and all such modifications are intended to be included within the scope of the following claims.

I claim:

1. A cytoprotective composition for preventing and reducing injury to mammalian cells from a medicament having cytotoxic properties which comprises:

(a) pyruvate selected from the group consisting of pyruvic acid, pharmaceutically acceptable salts of pyruvic acid, and mixtures thereof; and

(b) an antioxidant.

2. The cytoprotective composition according to claim 1, wherein the pyruvate is selected from the group consisting of pyruvic acid, sodium pyruvate, potassium pyruvate, magnesium pyruvate, calcium pyruvate, zinc pyruvate, manganese pyruvate, and mixtures thereof.

3. The cytoprotective composition according to claim 2, wherein the pyruvate is sodium pyruvate.

4. The cytoprotective composition according to claim 1, wherein the antioxidant is selected from the group consisting of retinol, 3, 4-didehydroretinol, *alpha*-carotene, *beta*-carotene, *gamma*-carotene, *delta*-carotene, ascorbic acid, *alpha*-tocopherol, *beta*-tocopherol, *gamma*-tocopherol, *delta*-tocopherol, and mixtures thereof.

5. The cytoprotective composition according to claim 4, wherein the antioxidant is *alpha*-tocopherol.

6. The cytoprotective composition according to claim 1, wherein pyruvate is present in the cytoprotective composition in an amount from about 10% to about 75%, by weight.

7. The cytoprotective composition according to claim 1, wherein the antioxidant is present in the cytoprotective composition in an amount from about 10% to about 75%, by weight.

8. A cytoprotective composition for preventing and reducing injury to mammalian cells from a medicament having cytotoxic properties which comprises:

(a) pyruvate selected from the group consisting of pyruvic acid, pharmaceutically acceptable salts of pyruvic acid, and mixtures thereof;

(b) an antioxidant; and

(c) a mixture of saturated and unsaturated fatty acids wherein the fatty acids are those fatty acids required for the repair of cellular membranes and resuscitation of mammalian cells.

9. The cytoprotective composition according to claim 8, wherein the mammalian cells comprise epidermal keratinocytes.

10. The cytoprotective composition according to claim 8, wherein the pyruvate is selected from the group consisting of pyruvic acid, sodium pyruvate, potassium pyruvate, magnesium pyruvate, calcium pyruvate, zinc pyruvate, manganese pyruvate, and mixtures thereof.

11. The cytoprotective composition according to claim 8, wherein the antioxidant is selected from the group consisting of retinol, 3, 4-didehydroretinol, *alpha*-carotene, *beta*-carotene, *gamma*-carotene, *delta*-carotene, ascorbic acid, *alpha*-tocopherol, *beta*-tocopherol, *gamma*-tocopherol, *delta*-tocopherol, and mixtures thereof.

12. The cytoprotective composition according to claim 8, wherein the mixture of saturated and unsaturated fatty acids comprises animal and vegetable fats and waxes.

13. The cytoprotective composition according to claim 12, wherein the mixture of saturated and unsaturated fatty acids comprises human fat, chicken fat, cow fat, sheep fat, horse fat, pig fat, and whale fat.

14. The cytoprotective composition according to claim 13, wherein the mixture of saturated and unsaturated fatty acids comprises lauric acid, myristic acid, myristoleic acid, pentadecanoic acid, palmitic acid, palmitoleic acid, margaric acid, margaroleic acid, stearic, oleic acid, linoleic acid, linolenic acid, arachidic acid, and gaddoleic acid.

15. The cytoprotective composition according to claim 8, wherein pyruvate is present in the cytoprotective composition in an amount from about 10% to about 50%, by weight.

16. The cytoprotective composition according to claim 8, wherein the antioxidant is present in the cytoprotective composition in an amount from about 10% to about 50%, by weight.

17. The cytoprotective composition according to claim 8, wherein the mixture of saturated and unsaturated fatty acids is present in the cytoprotective composition in an amount from about 10% to about 50%, by weight.

18. A cytoprotective pharmaceutical composition for preventing and reducing injury to mammalian cells from a medicament having cytotoxic properties which comprises:

(A) a cytoprotective composition selected from the group consisting of:

(1) (a) pyruvate selected from the group consisting of pyruvic acid, pharmaceutically acceptable salts of pyruvic acid, and mixtures thereof;

(b) an antioxidant; and

(c) a mixture of saturated and unsaturated fatty acids wherein the fatty acids are those fatty acids required for the resuscitation of injured mammalian cells; and

(2) (a) pyruvate selected from the group consisting of pyruvic acid, pharmaceutically acceptable salts of pyruvic acid, and mixtures thereof; and

(b) an antioxidant; and

(B) a medicament having cytotoxic properties.

19. A timed-release cytoprotective pharmaceutical composition for selectively protecting non-cancerous mammalian cells in the presence of cancerous mammalian cells from an anticancer medicament having cytotoxic properties which comprises:

(A) a cytoprotective composition in immediate release form selected from the group consisting of:

(1) (a) pyruvate selected from the group consisting of pyruvic acid, pharmaceutically acceptable salts of pyruvic acid, and mixtures thereof;

(b) an antioxidant; and

(c) a mixture of saturated and unsaturated fatty acids wherein the fatty acids are those fatty acids required for the resuscitation of injured mammalian cells; and

(2) (a) pyruvate selected from the group consisting of pyruvic acid, pharmaceutically acceptable salts of pyruvic acid, and mixtures thereof; and

(b) an antioxidant; and

(B) an anticancer medicament having cytotoxic properties in timed-release form;

wherein the cytoprotective composition is released substantially immediately and the anticancer medicament is released after a period of time sufficient such that the cancerous cells have substantially metabolized the cytoprotective composition and the non-cancerous cells have not substantially metabolized the cytoprotective composition.

20. A method for preparing a cytoprotective composition for preventing and reducing injury to mammalian cells from a medicament having cytotoxic properties which comprises the steps of admixing the following ingredients:

(a) pyruvate selected from the group consisting of pyruvic acid, pharmaceutically acceptable salts of pyruvic acid, and mixtures thereof; and

(b) an antioxidant.

21. A method for preparing a cytoprotective composition for preventing and reducing injury to mammalian cells from a medicament having cytotoxic properties which comprises the steps of admixing the following ingredients:

(a) pyruvate selected from the group consisting of pyruvic acid, pharmaceutically acceptable salts of pyruvic acid, and mixtures thereof;

(b) an antioxidant; and

(c) a mixture of saturated and unsaturated fatty acids wherein the fatty acids are those fatty acids required for the repair of cellular membranes and resuscitation of mammalian cells.

22. A method for preparing a cytoprotective pharmaceutical composition for preventing and reducing injury to mammalian cells from a medicament having cytotoxic properties which comprises the steps of:

(A) providing a cytoprotective composition selected from the group consisting of:

(1) (a) pyruvate selected from the group consisting of pyruvic acid, pharmaceutically acceptable salts of pyruvic acid, and mixtures thereof;

(b) an antioxidant; and

(c) a mixture of saturated and unsaturated fatty acids wherein the fatty acids are those fatty acids required for the resuscitation of injured mammalian cells; and

(2) (a) pyruvate selected from the group consisting of pyruvic acid, pharmaceutically acceptable salts of pyruvic acid, and mixtures thereof; and

(b) an antioxidant; and

(B) providing an anticancer medicament having cytotoxic properties; and

(C) admixing the cytoprotective composition from step (A) with the medicament from step (B) to prepare the cytoprotective pharmaceutical composition.

23. A method for preparing a timed-release cytoprotective pharmaceutical composition for selectively protecting non-cancerous mammalian cells in the presence of cancerous mammalian cells from an anticancer medicament having cytotoxic properties which comprises the steps of:

(A) providing a cytoprotective composition in an immediate release form selected from the group consisting of:

(1) (a) pyruvate selected from the group consisting of pyruvic acid, pharmaceutically acceptable salts of pyruvic acid, and mixtures thereof;

(b) an antioxidant; and

(c) a mixture of saturated and unsaturated fatty acids wherein the fatty acids are those fatty acids required for the resuscitation of injured mammalian cells; and

(2) (a) pyruvate selected from the group consisting of pyruvic acid, pharmaceutically acceptable salts of pyruvic acid, and mixtures thereof; and

(b) an antioxidant; and

(B) providing an anticancer medicament having cytotoxic properties in timed-release form; and

(C) admixing the cytoprotective composition from step (A) with the medicament from step (B) to prepare the timed-release cytoprotective pharmaceutical composition;

wherein the cytoprotective composition is released substantially immediately and the anticancer medicament is released after a period of time sufficient such that the cancerous cells have substantially metabolized the cytoprotective composition and the non-cancerous cells have not substantially metabolized the cytoprotective composition.

24. A method for protecting mammalian cells from a medicament having cytotoxic properties which comprises the steps of:

(A) providing a cytoprotective composition selected from the group consisting of:

(1) (a) pyruvate selected from the group consisting of pyruvic acid, pharmaceutically acceptable salts of pyruvic acid, and mixtures thereof;

(b) an antioxidant; and

(c) a mixture of saturated and unsaturated fatty acids wherein the fatty acids are those fatty acids required for the resuscitation of injured mammalian cells; and

(2) (a) pyruvate selected from the group consisting of pyruvic acid, pharmaceutically acceptable salts of pyruvic acid, and mixtures thereof; and

(b) an antioxidant; and

(B) providing an anticancer medicament having cytotoxic properties; and

(C) administering the cytoprotective composition from step (A) and the medicament from step (B) concurrently to mammalian cells to protect the mammalian cells from the medicament having cytotoxic properties.

25. A method for selectively protecting non-cancerous mammalian cells in the presence of cancerous mammalian cells from an anticancer medicament having cytotoxic properties which comprises the steps of:

(A) providing a cytoprotective composition in an immediate release form selected from the group consisting of:

(1) (a) pyruvate selected from the group consisting of pyruvic acid, pharmaceutically acceptable salts of pyruvic acid, and mixtures thereof;

(b) an antioxidant; and

(c) a mixture of saturated and unsaturated fatty acids wherein the fatty acids are those fatty acids required for the resuscitation of injured mammalian cells; and

(2) (a) pyruvate selected from the group consisting of pyruvic acid, pharmaceutically acceptable salts of pyruvic acid, and mixtures thereof; and

(b) an antioxidant; and

(B) providing an anticancer medicament having cytotoxic properties in timed-release form; and

(C) administering the cytoprotective composition from step (A) and the medicament from step (B) concurrently to mammalian cells to selectively protect non-cancerous mammalian cells in the presence of cancerous mammalian cells from the anticancer medicament having cytotoxic properties;

wherein the cytoprotective composition is released substantially immediately and the anticancer medicament is released after a period of time sufficient such that the cancerous cells have substantially metabolized the cytoprotective composition and the non-cancerous cells have not substantially metabolized the cytoprotective composition.

26. A method for selectively protecting non-cancerous mammalian cells in the presence of cancerous mammalian cells from an anticancer medicament having cytotoxic properties which comprises the steps of:

(A) administering to mammalian cells a cytoprotective composition to prevent and reduce injury to the mammalian cells selected from the group consisting of:

(1) (a) pyruvate selected from the group consisting of pyruvic acid, pharmaceutically acceptable salts of pyruvic acid, and mixtures thereof;

(b) an antioxidant; and

(c) a mixture of saturated and unsaturated fatty acids wherein the fatty acids are those fatty acids required for the resuscitation of injured mammalian cells; and

(2) (a) pyruvate selected from the group consisting of pyruvic acid, pharmaceutically acceptable salts of pyruvic acid, and mixtures thereof; and

(b) an antioxidant; and

(B) waiting a period of time sufficient such that the cancerous cells have substantially metabolized the cytoprotective composition and the non-cancerous cells have not substantially metabolized the cytoprotective composition; and

(C) administering the cytotoxic anticancer medicament to the mammalian cells to treat the cancerous cells which are unprotected by the cytoprotective composition and the non-cancerous cells which are protected by the cytoprotective composition to thereby increase the therapeutic effect of the anticancer medicament.

27. A cytoprotective pharmaceutical composition which comprises a pharmaceutically acceptable carrier and a therapeutically effective amount of a cytoprotective composition for preventing and reducing injury to mammalian cells from a medicament having cytotoxic properties, wherein the cytoprotective composition is selected from the group consisting of:

(1) (a) pyruvate selected from the group consisting of pyruvic acid, pharmaceutically acceptable salts of pyruvic acid, and mixtures thereof;

(b) an antioxidant; and

(c) a mixture of saturated and unsaturated fatty acids wherein the fatty acids are those fatty acids required for the resuscitation of injured mammalian cells; and

FIG. 1A

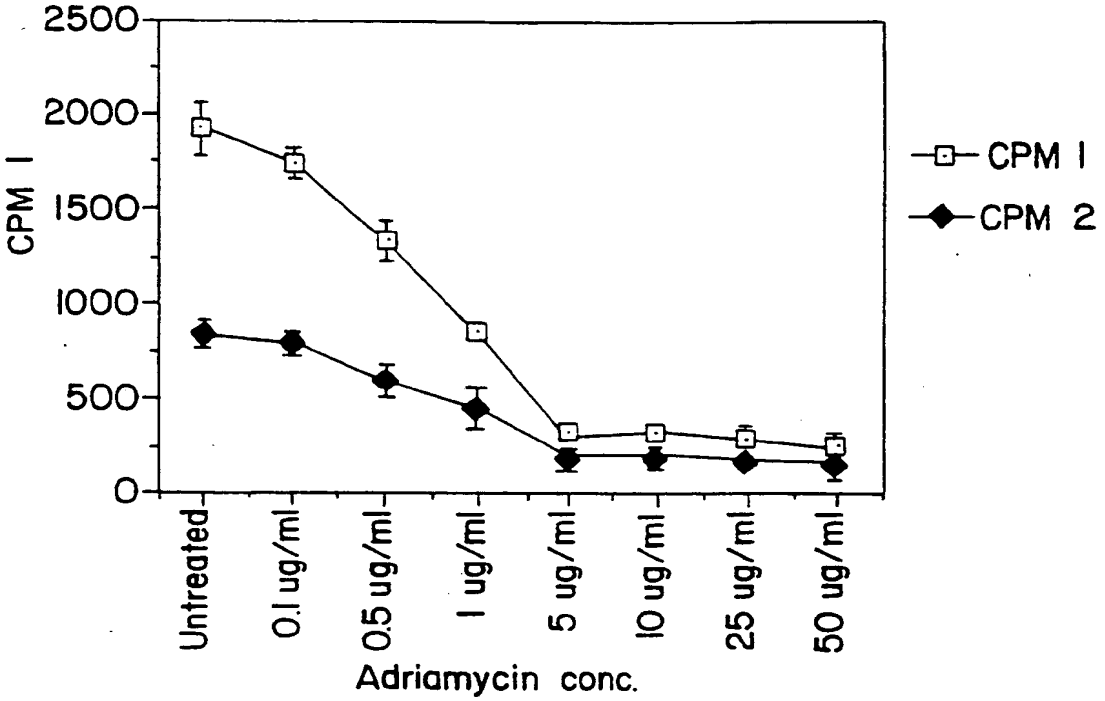

2500
2000
1500
1000
500
0
CPM 1
CPM 1
CPM 2
Untreated
0.1 ug/ml
0.5 ug/ml
1 ug/ml
5 ug/ml
10 ug/ml
25 ug/ml
50 ug/ml
Adriamycin conc.

FIG. 1B

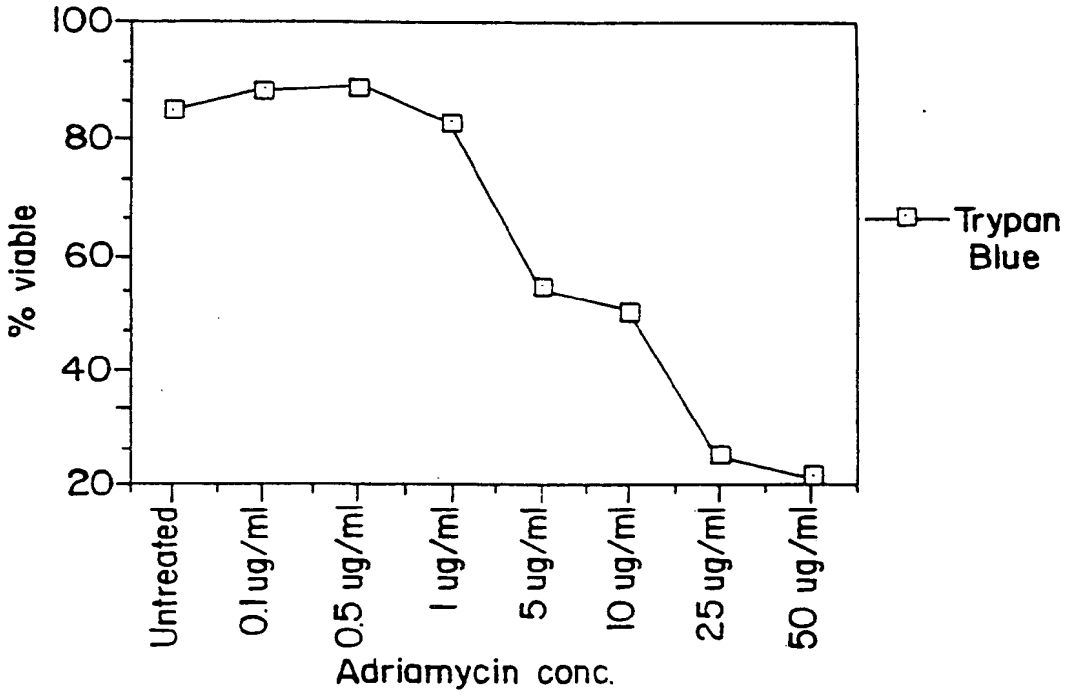

100
80
60
40
20
% viable
Trypan Blue
Untreated
0.1 ug/ml
0.5 ug/ml
1 ug/ml
5 ug/ml
10 ug/ml
25 ug/ml
50 ug/ml
Adriamycin conc.

FIG. 2

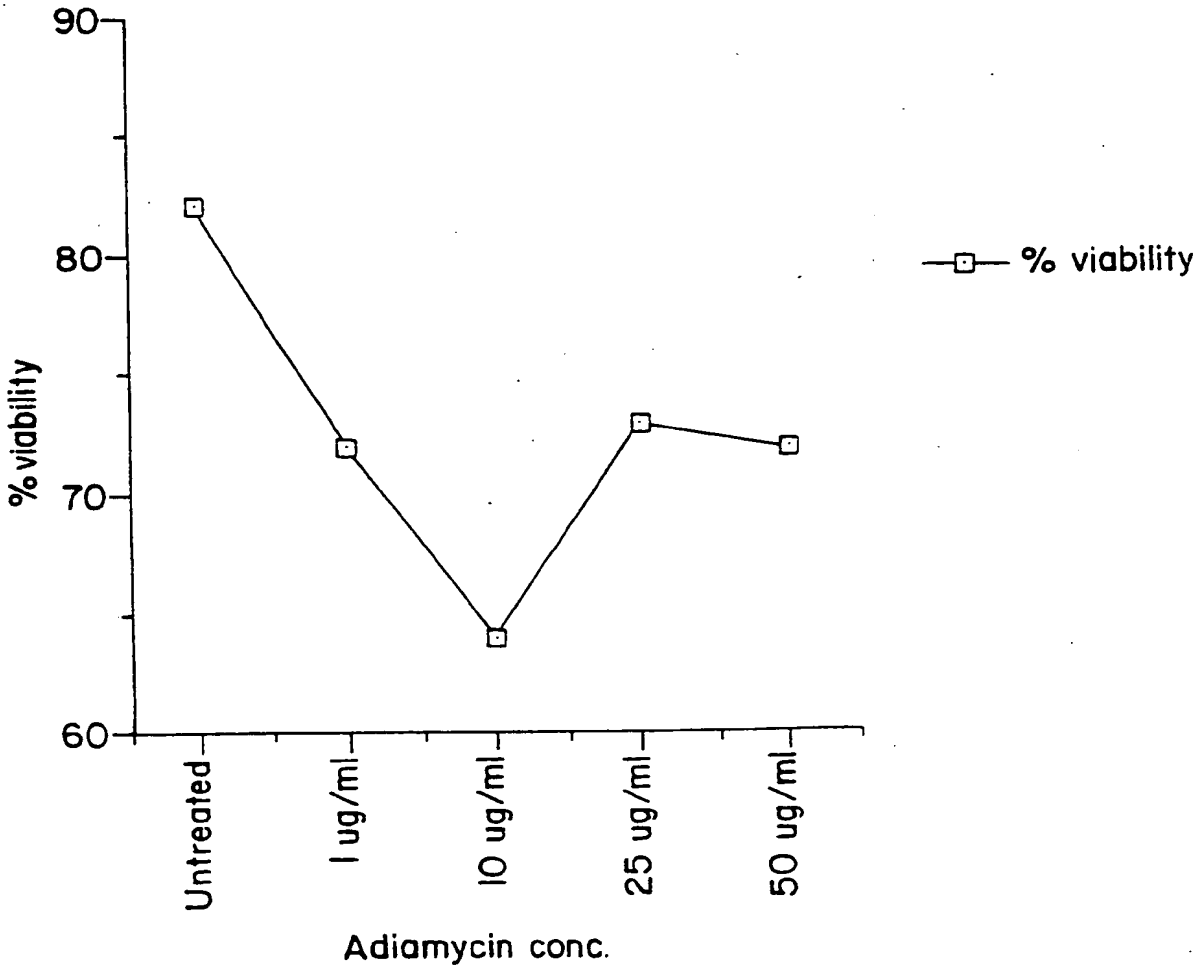


FIG. 3

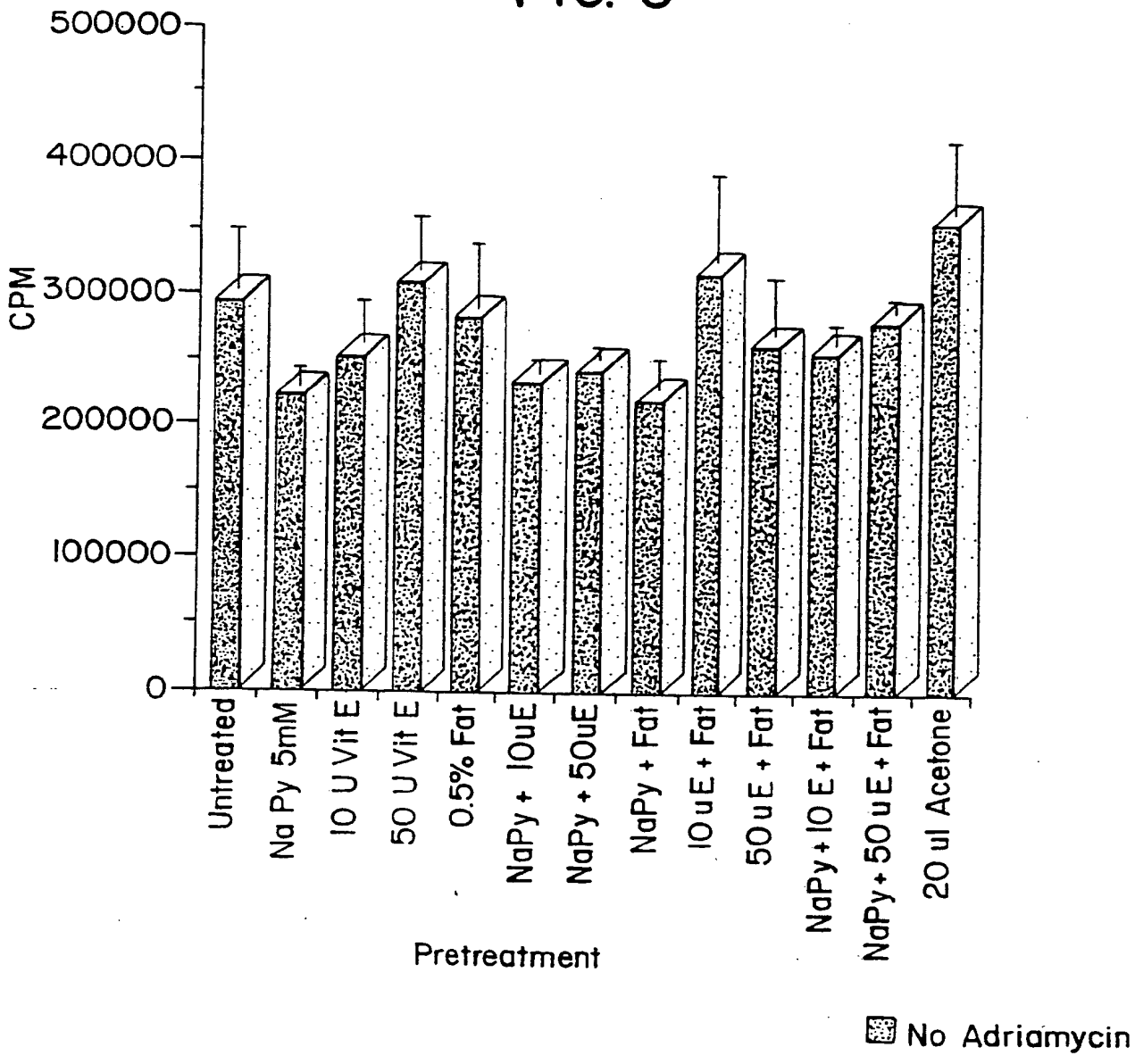

500000
400000
300000
200000
100000
0
CPM
Untreated
Na Py 5mM
10 U Vit E
50 U Vit E
0.5% Fat
NaPy + 10uE
NaPy + 50uE
NaPy + Fat
10uE + Fat
50uE + Fat
NaPy+10E+Fat
NaPy+50uE+Fat
20 ul Acetone
Pretreatment
No Adriamycin

FIG. 4A

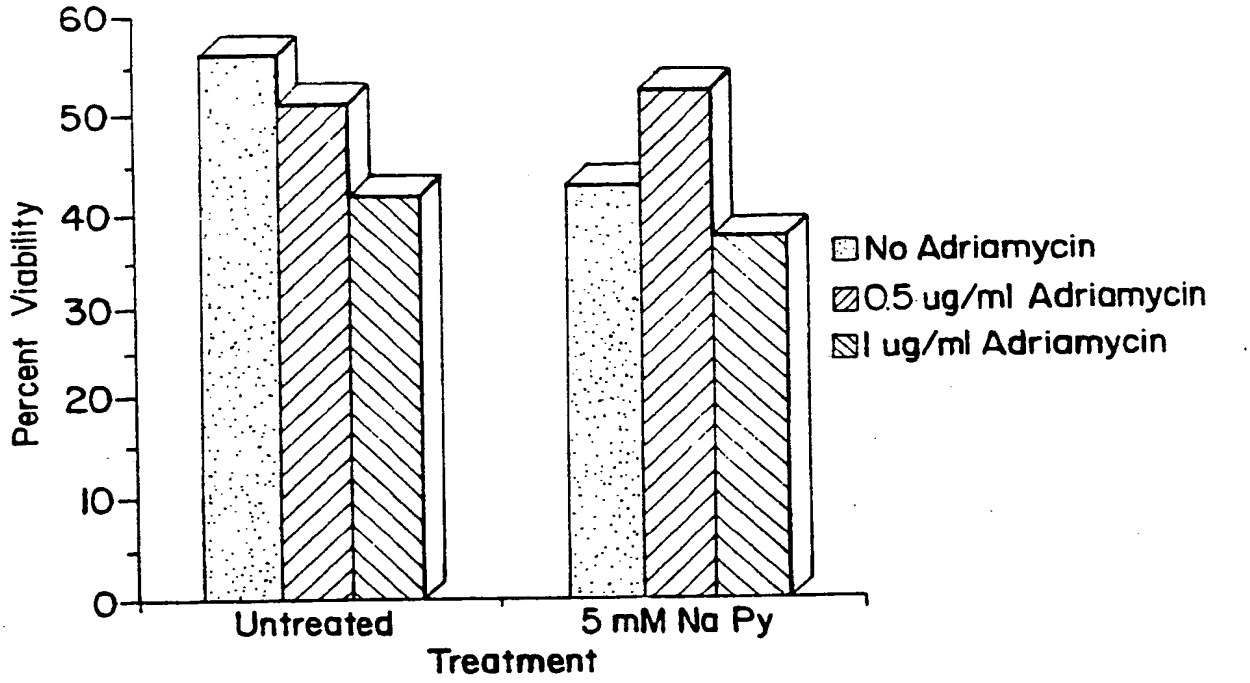


FIG. 4B

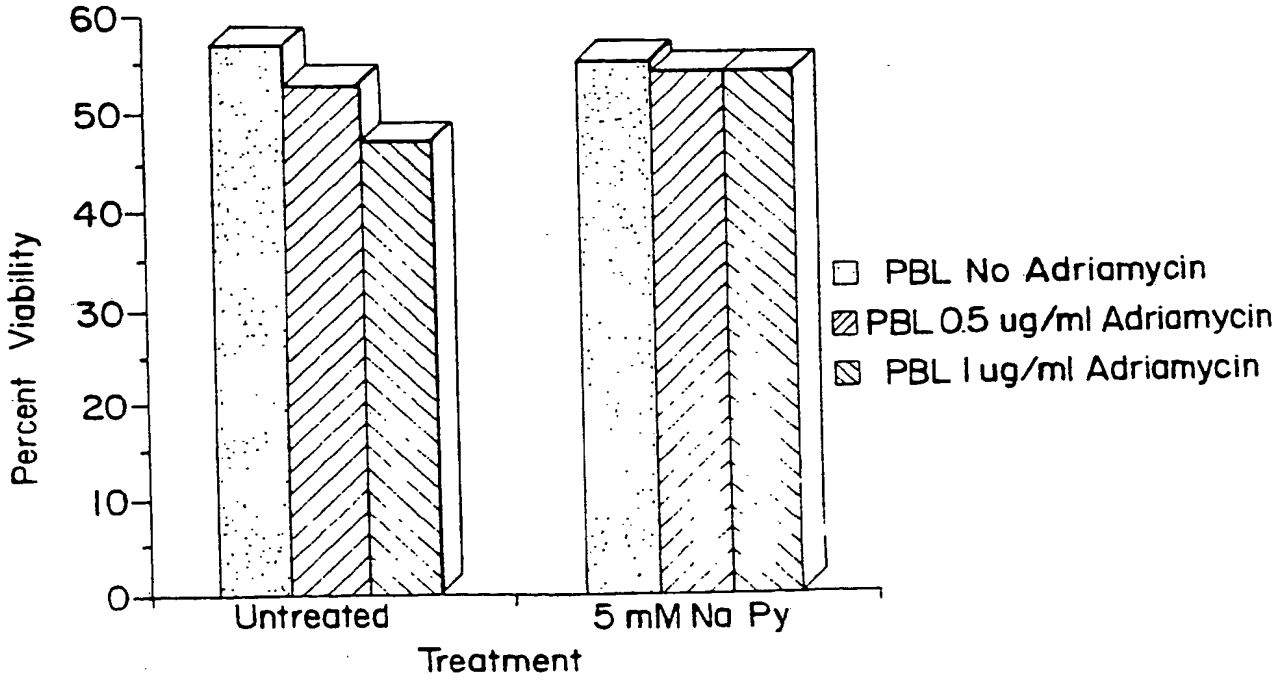


FIG. 5A

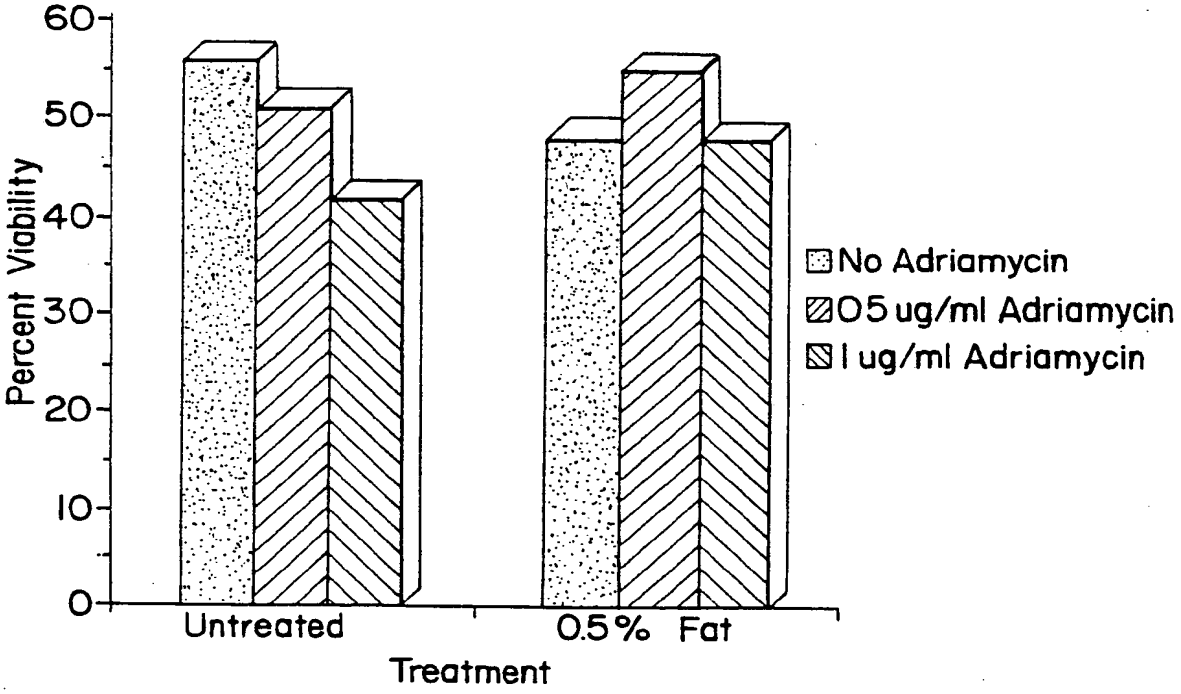

60
50
40
30
20
10
0
Percent Viability
Untreated
0.5% Fat
Treatment
No Adriamycin
0.5 ug/ml Adriamycin
1 ug/ml Adriamycin

FIG. 5B

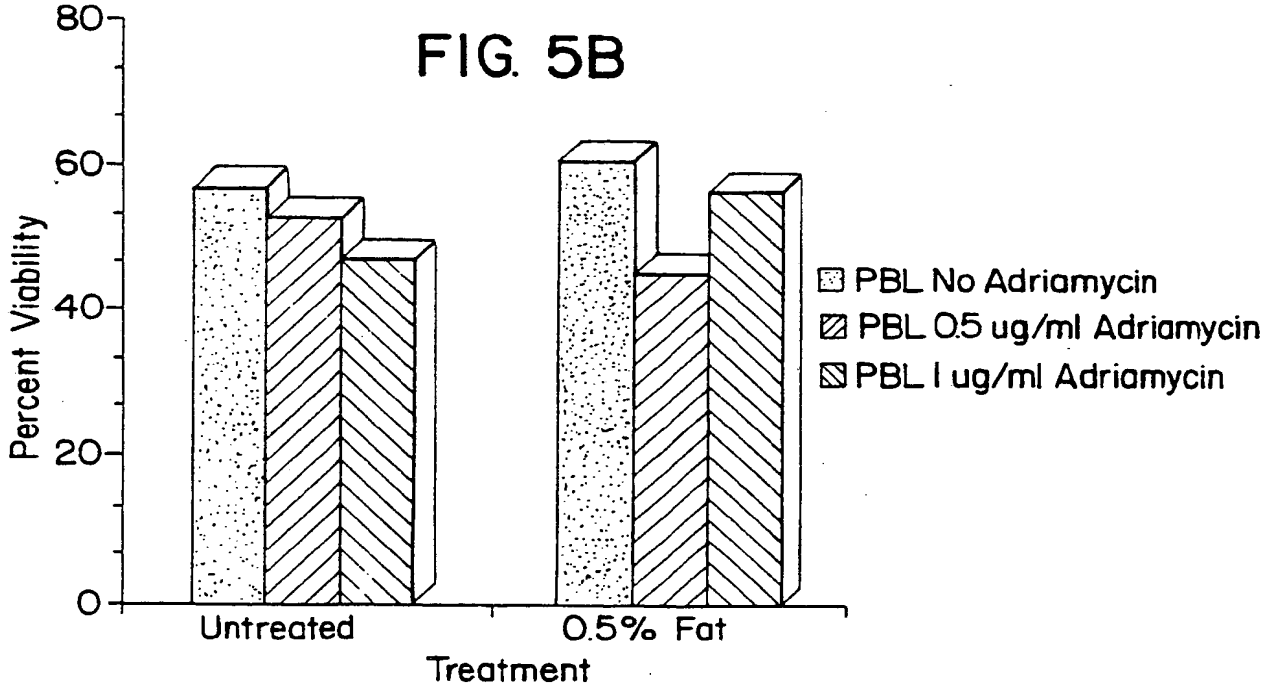

80
60
40
20
0
Percent Viability
Untreated
0.5% Fat
Treatment
PBL No Adriamycin
PBL 0.5 ug/ml Adriamycin
PBL 1 ug/ml Adriamycin

FIG. 6A

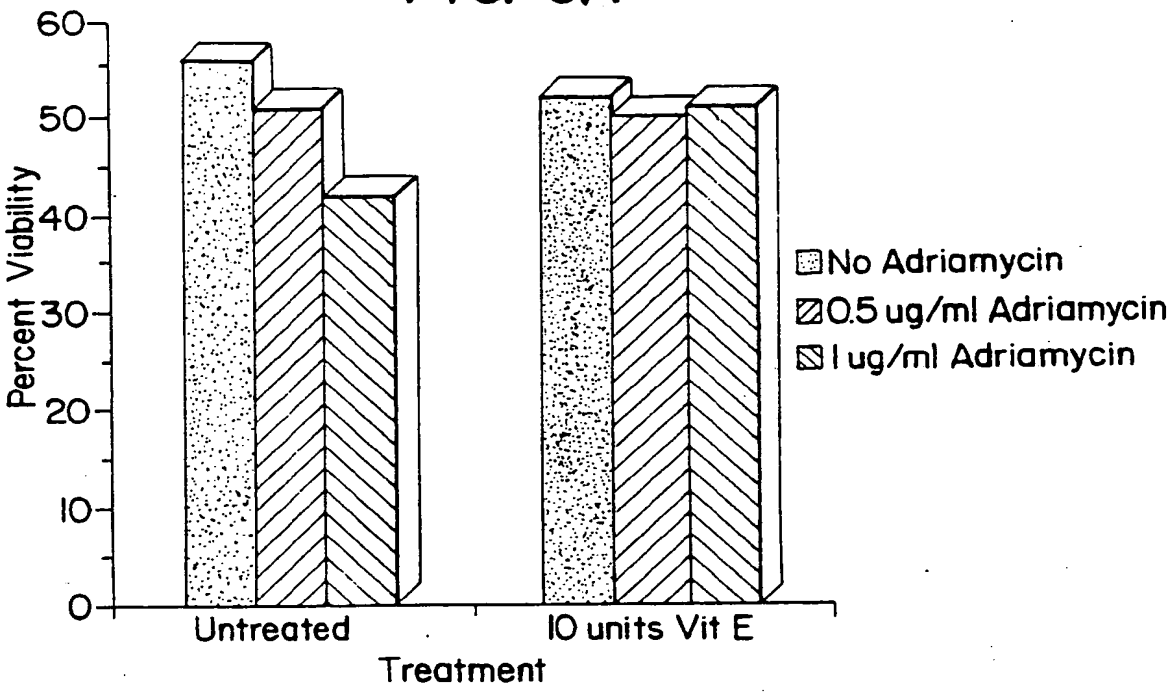

60
50
40
30
20
10
0
Percent Viability
Untreated
10 units Vit E
Treatment
No Adriamycin
0.5 ug/ml Adriamycin
1 ug/ml Adriamycin

FIG. 6B

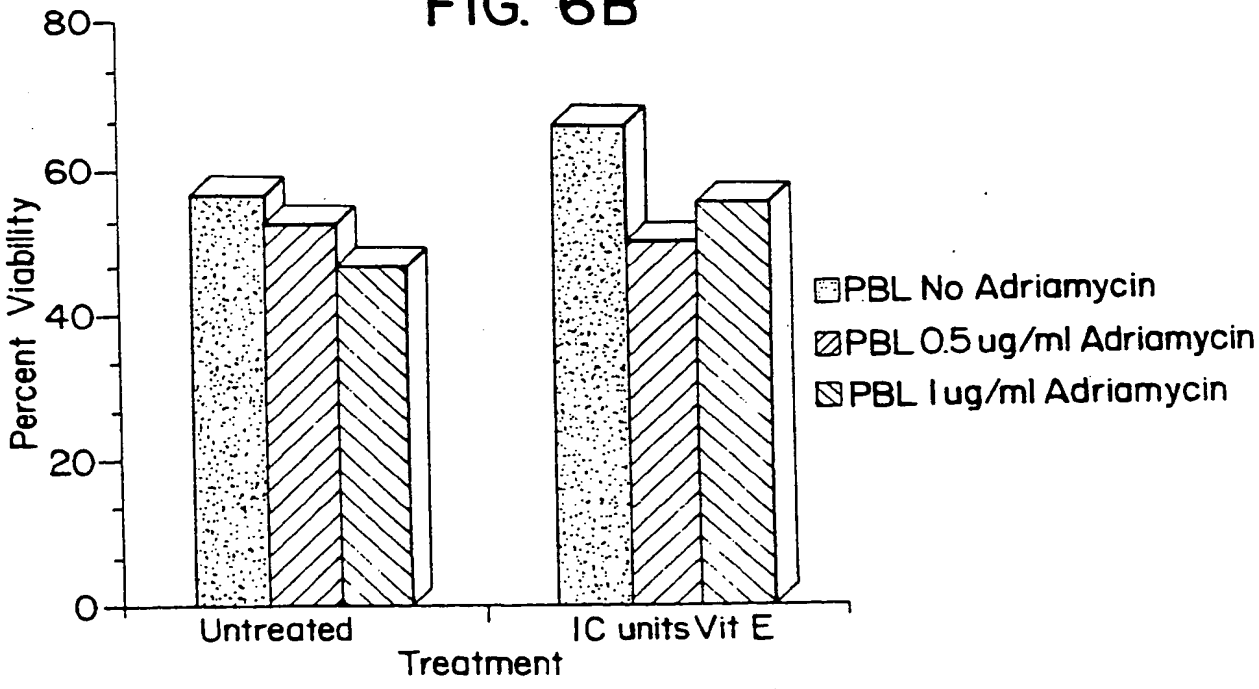

80
60
40
20
0
Percent Viability
Untreated
1C units Vit E
Treatment
PBL No Adriamycin
PBL 0.5 ug/ml Adriamycin
PBL 1 ug/ml Adriamycin

FIG. 7A

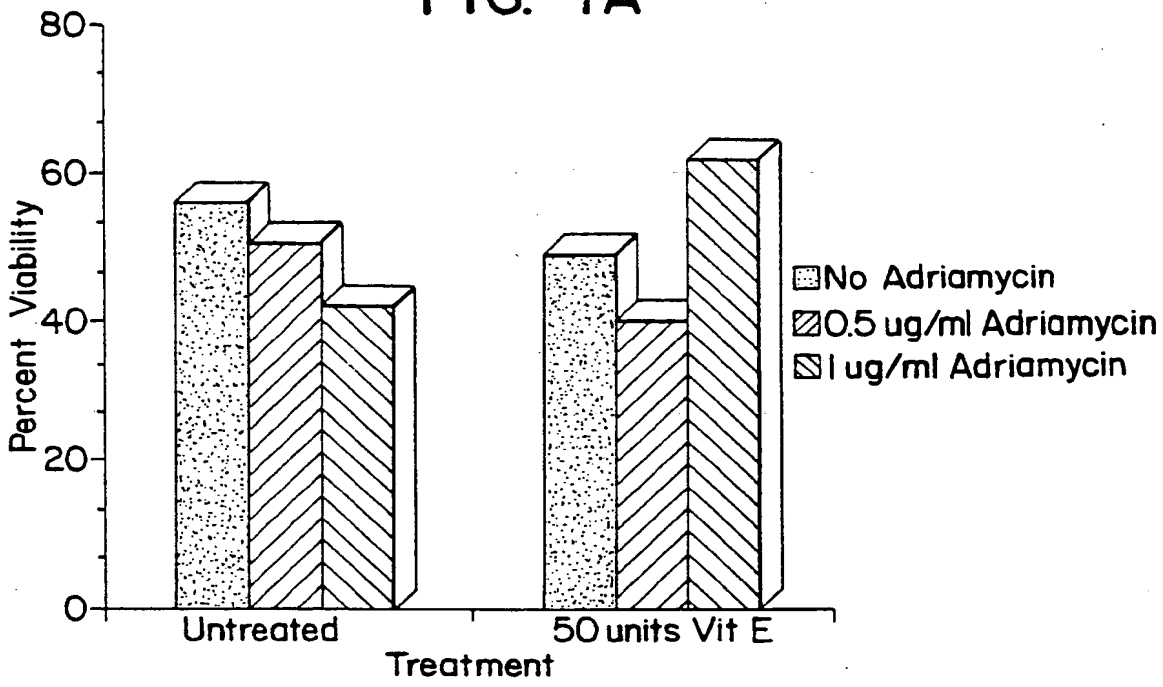

80
60
40
20
0
Percent Viability
Untreated
50 units Vit E
Treatment
No Adriamycin
0.5 ug/ml Adriamycin
1 ug/ml Adriamycin

FIG. 7B

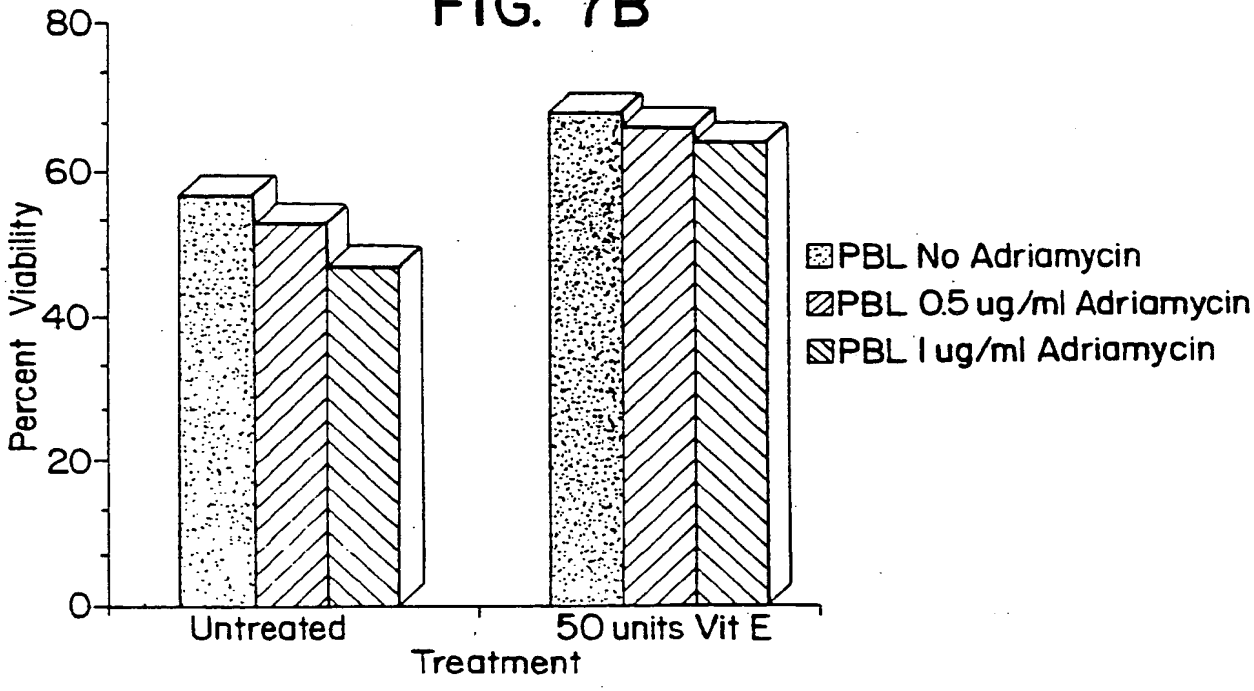

80
60
40
20
0
Percent Viability
Untreated
50 units Vit E
Treatment
PBL No Adriamycin
PBL 0.5 ug/ml Adriamycin
PBL 1 ug/ml Adriamycin

FIG. 8A

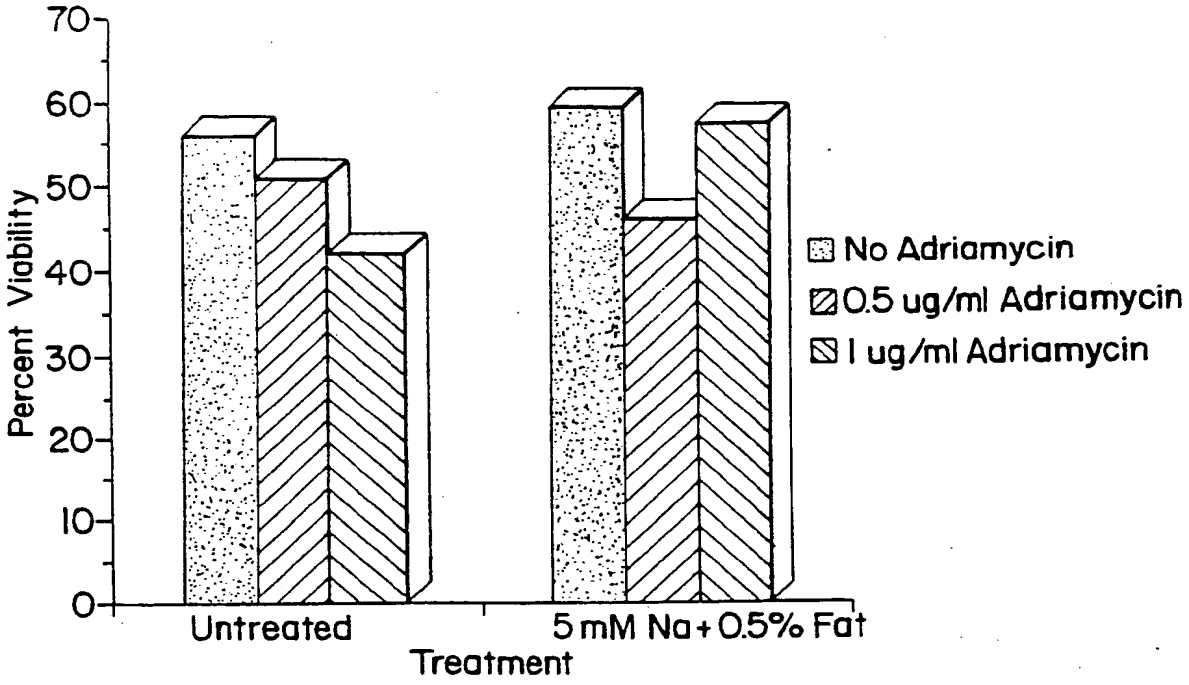


FIG. 8B

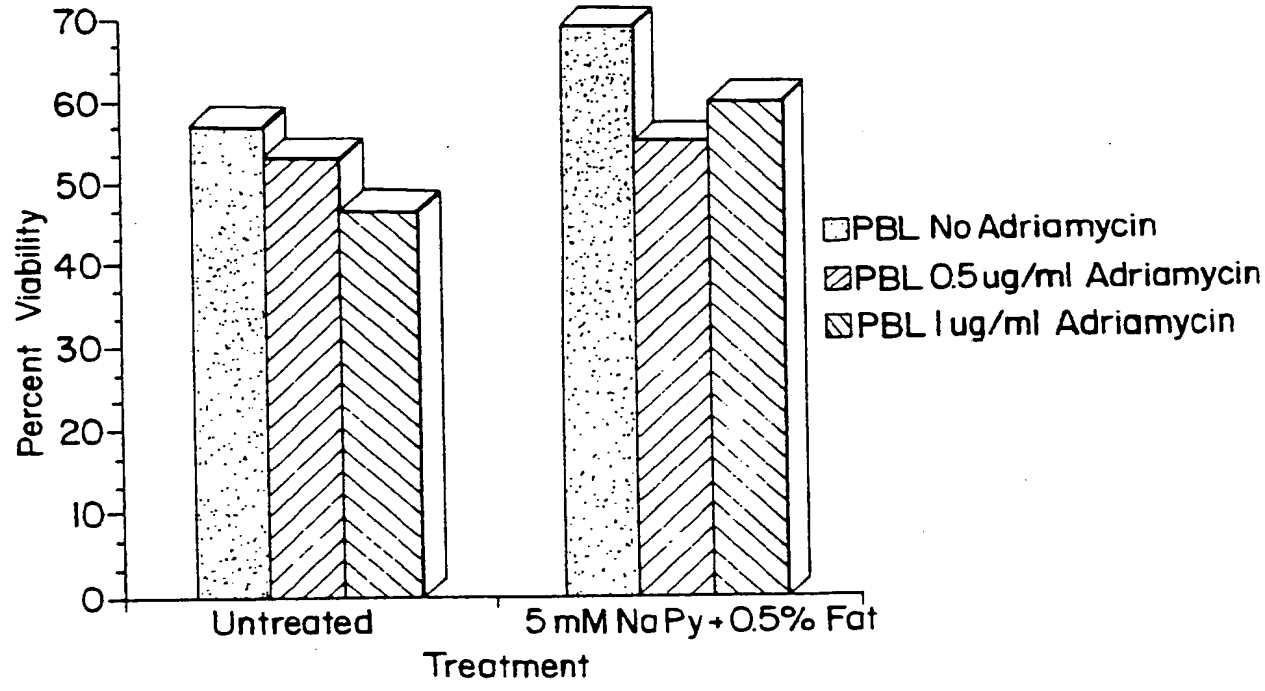


FIG. 9A

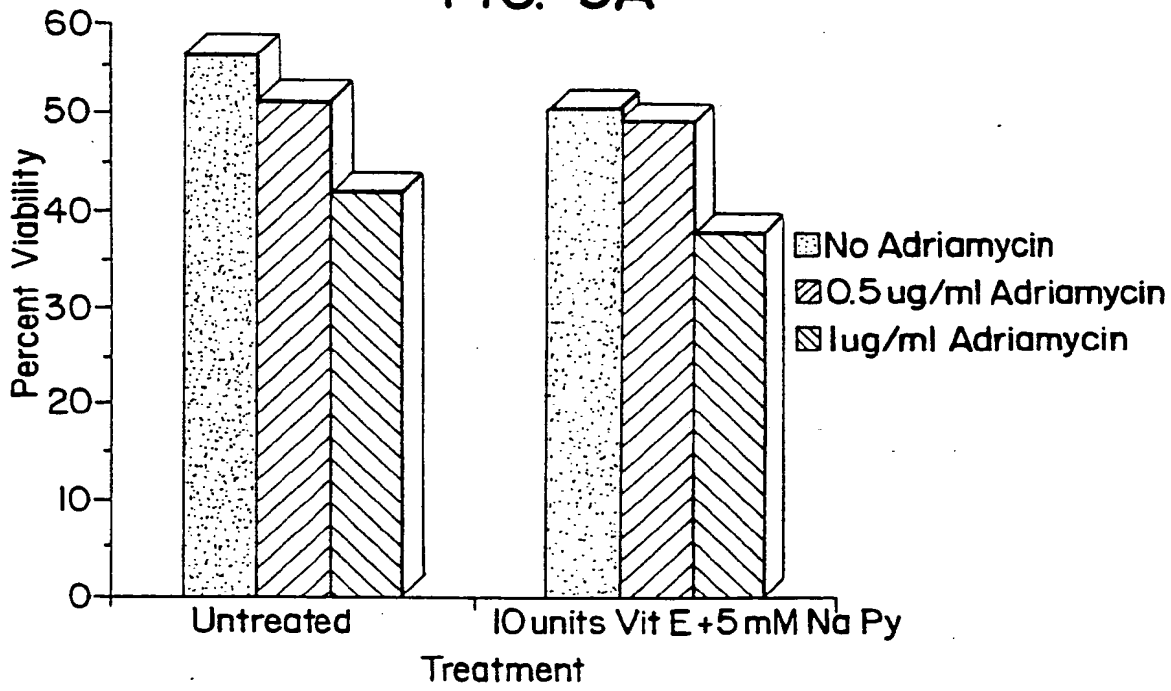

60
50
40
30
20
10
0
Percent Viability
No Adriamycin
0.5 ug/ml Adriamycin
1ug/ml Adriamycin
Untreated
10 units Vit E +5 mM Na Py
Treatment

FIG. 9B

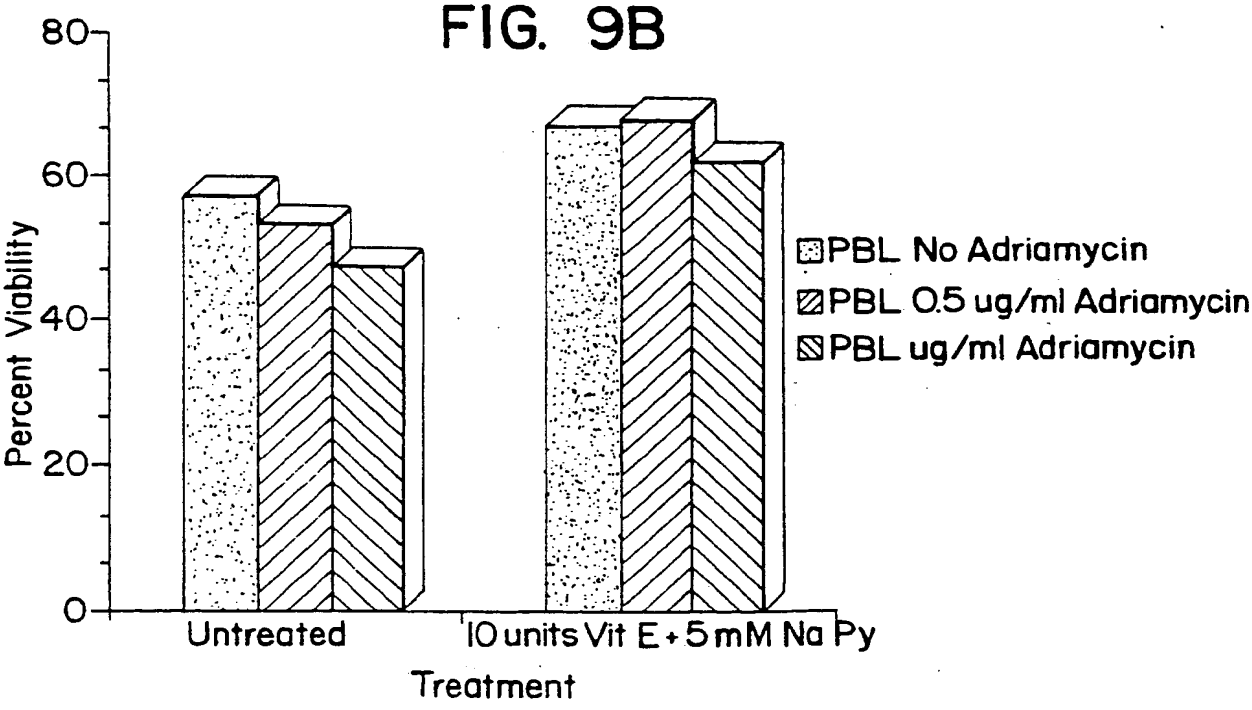

80
60
40
20
0
Percent Viability
PBL No Adriamycin
PBL 0.5 ug/ml Adriamycin
PBL ug/ml Adriamycin
Untreated
10 units Vit E + 5 mM Na Py
Treatment

FIG. 10A

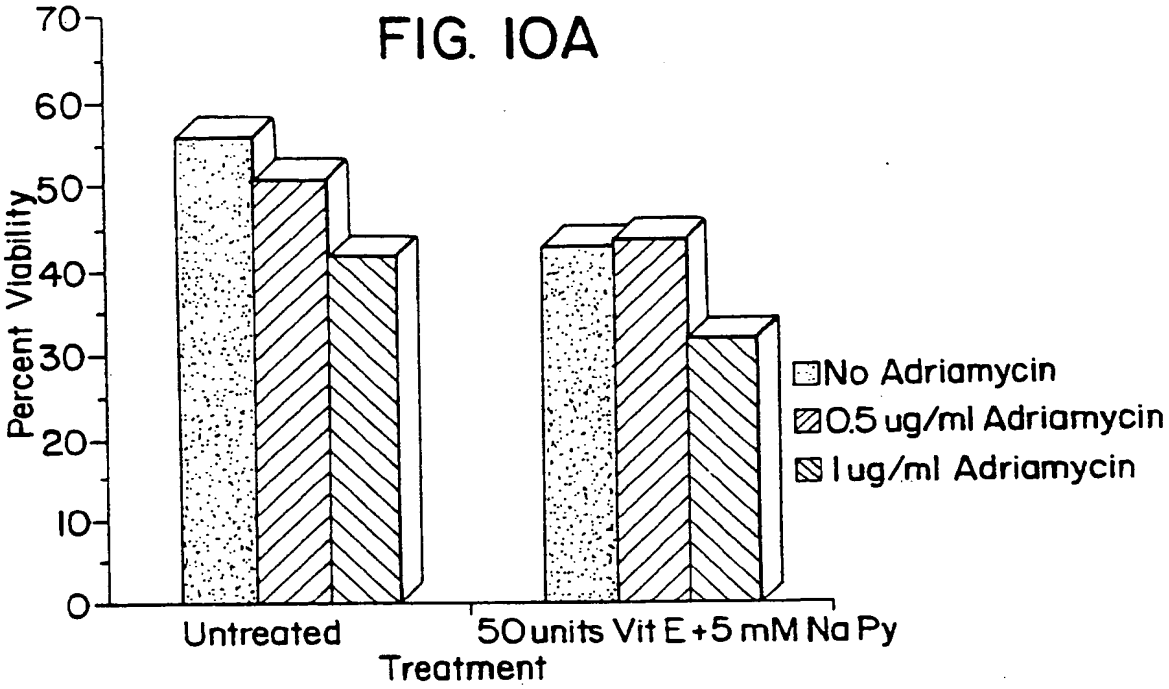


FIG. 10B

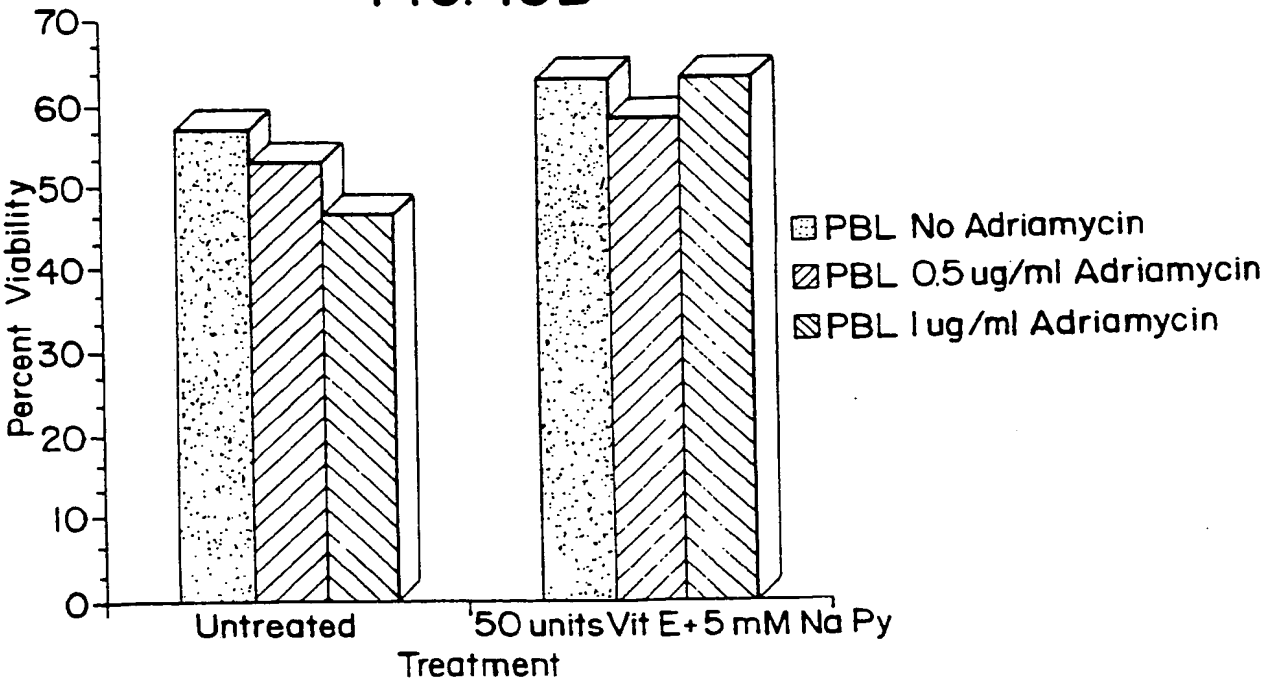


FIG. IIA

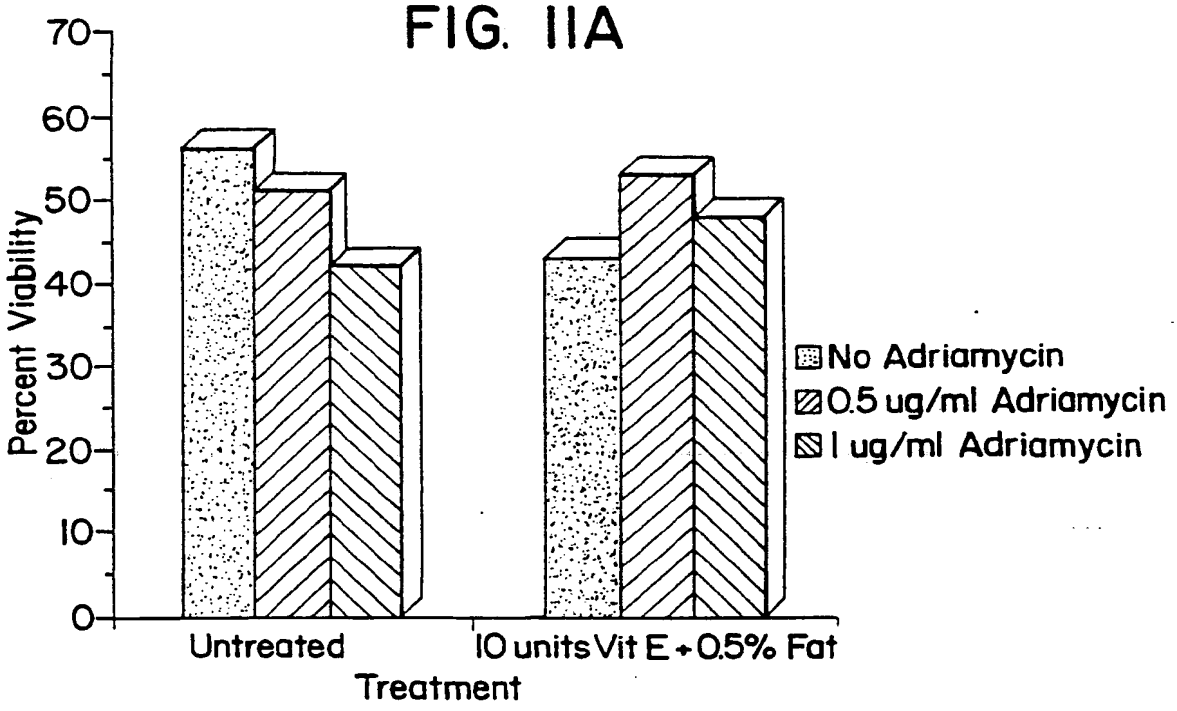


FIG. IIB

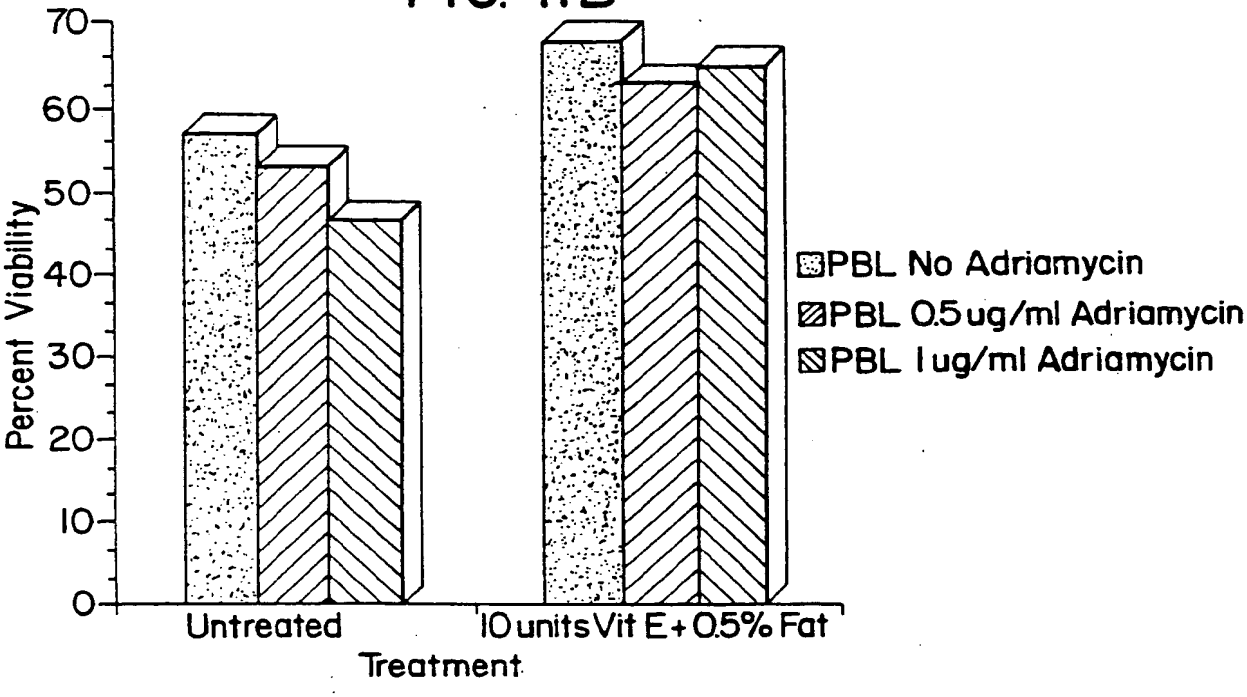


FIG. 12A

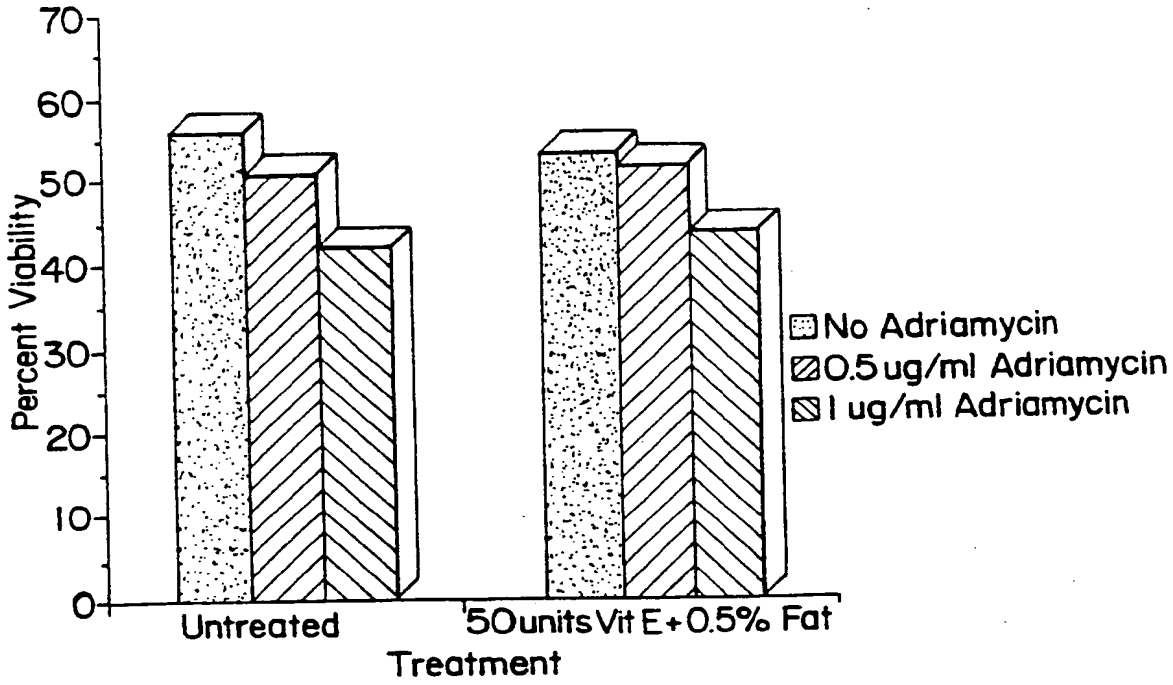


FIG. 12B

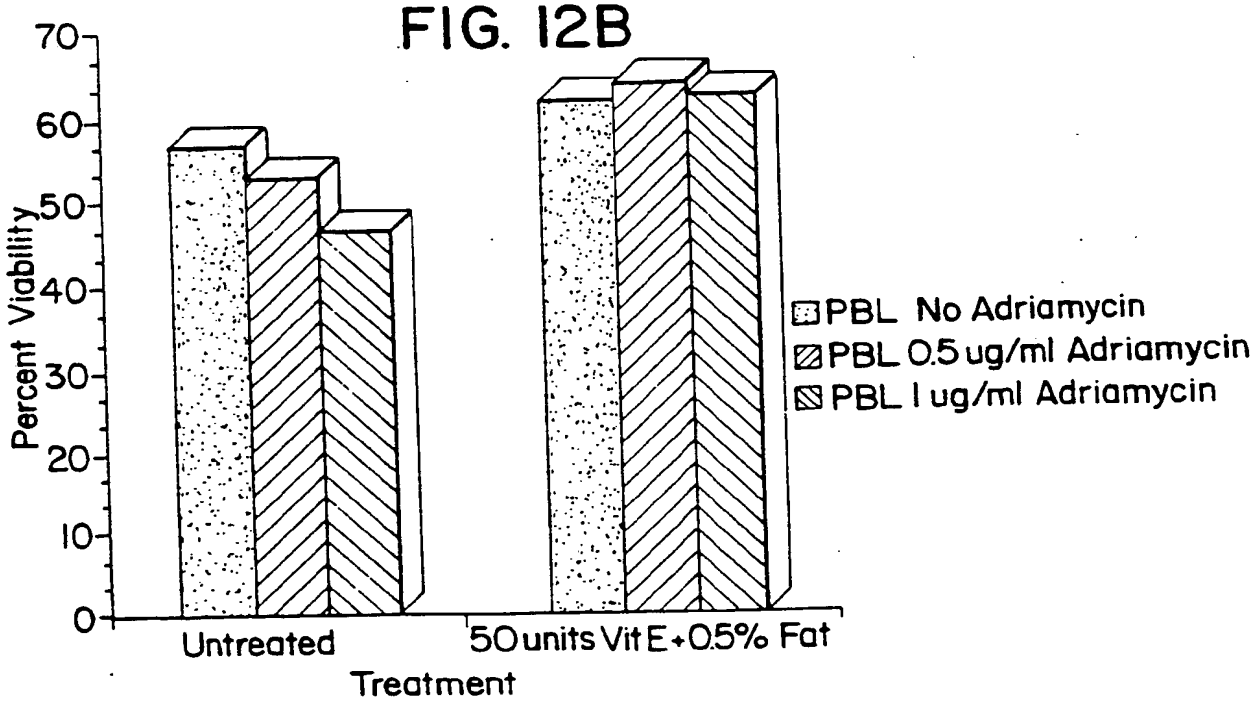


FIG. 13A

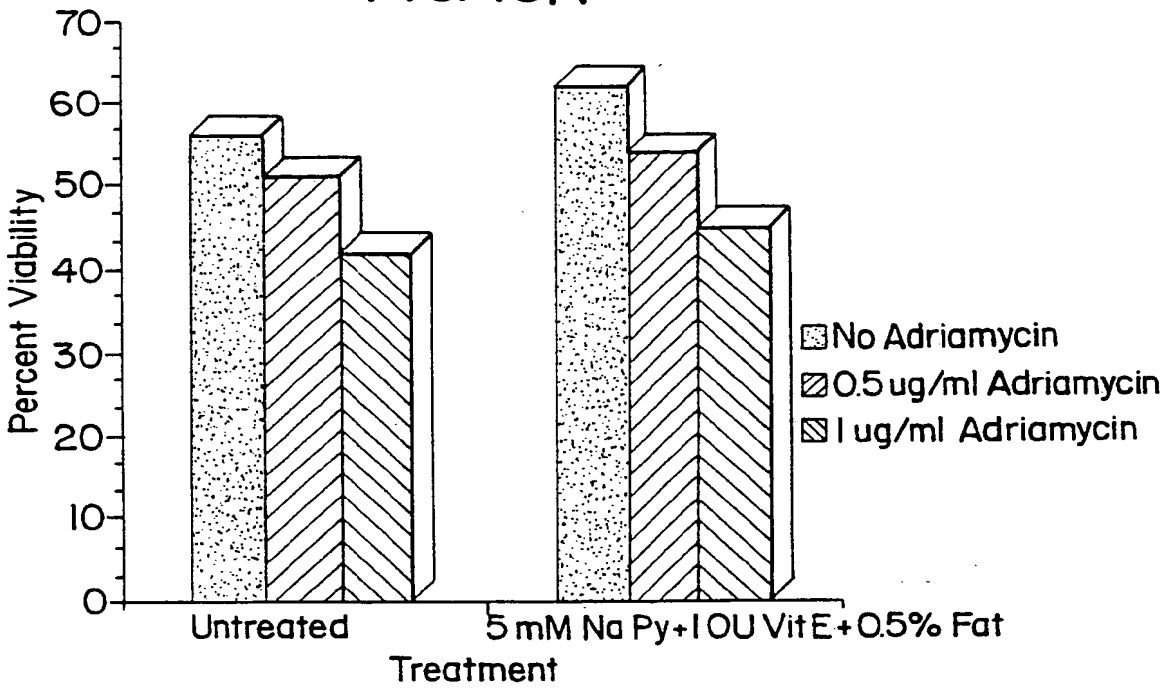


FIG. 13B

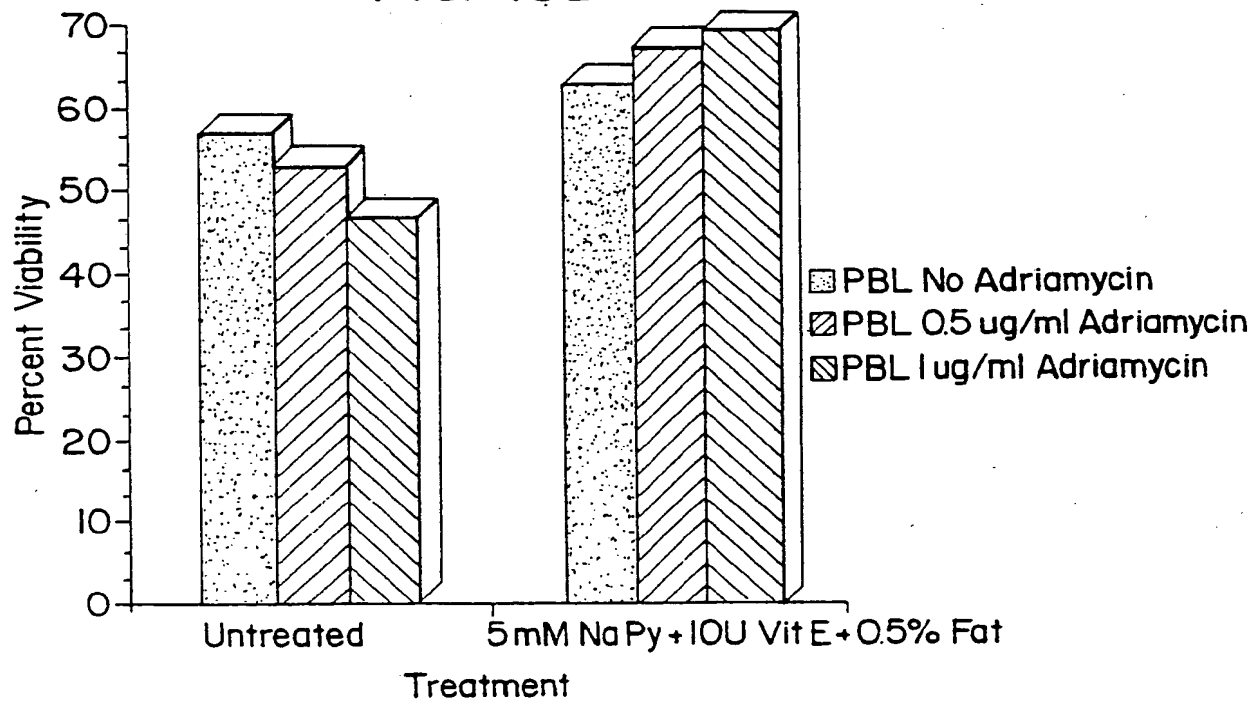


FIG. 14A

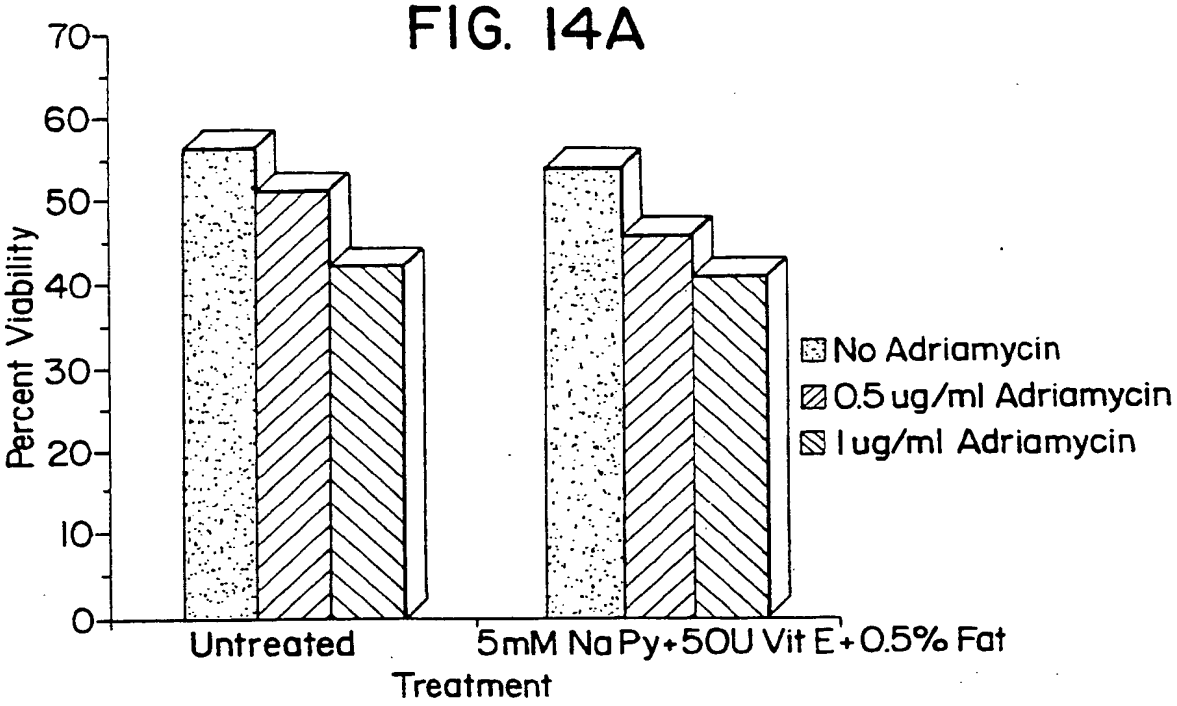


FIG. 14B

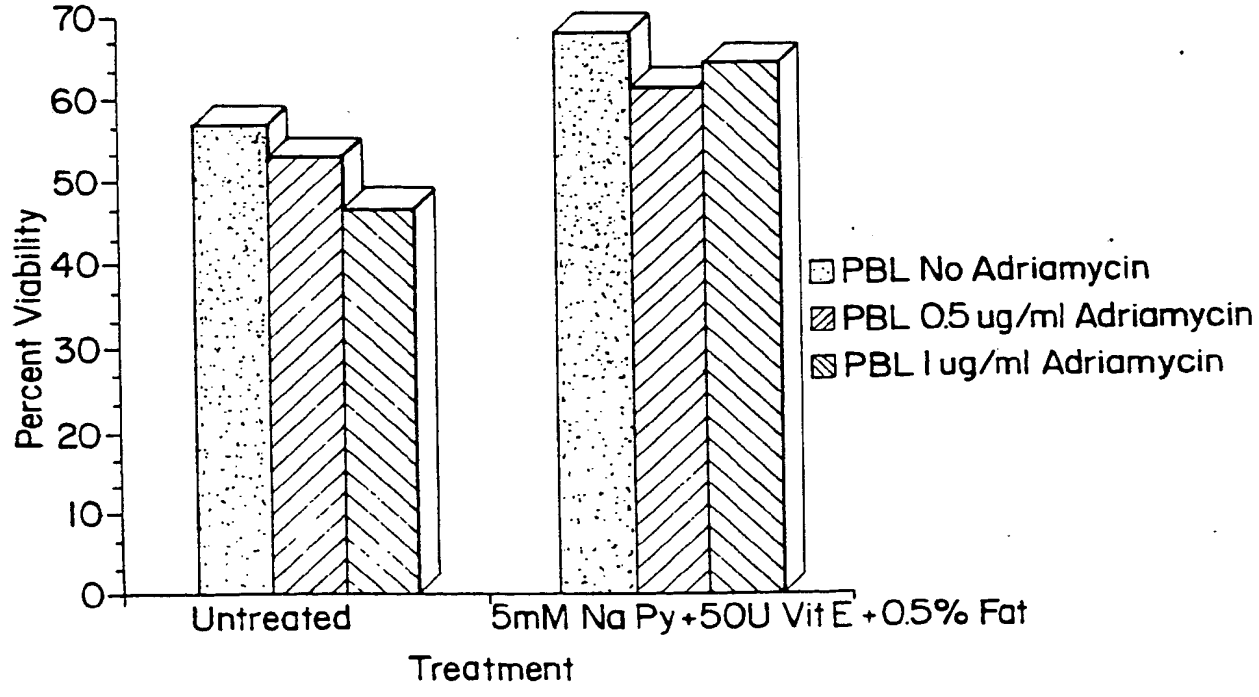


FIG. 15A

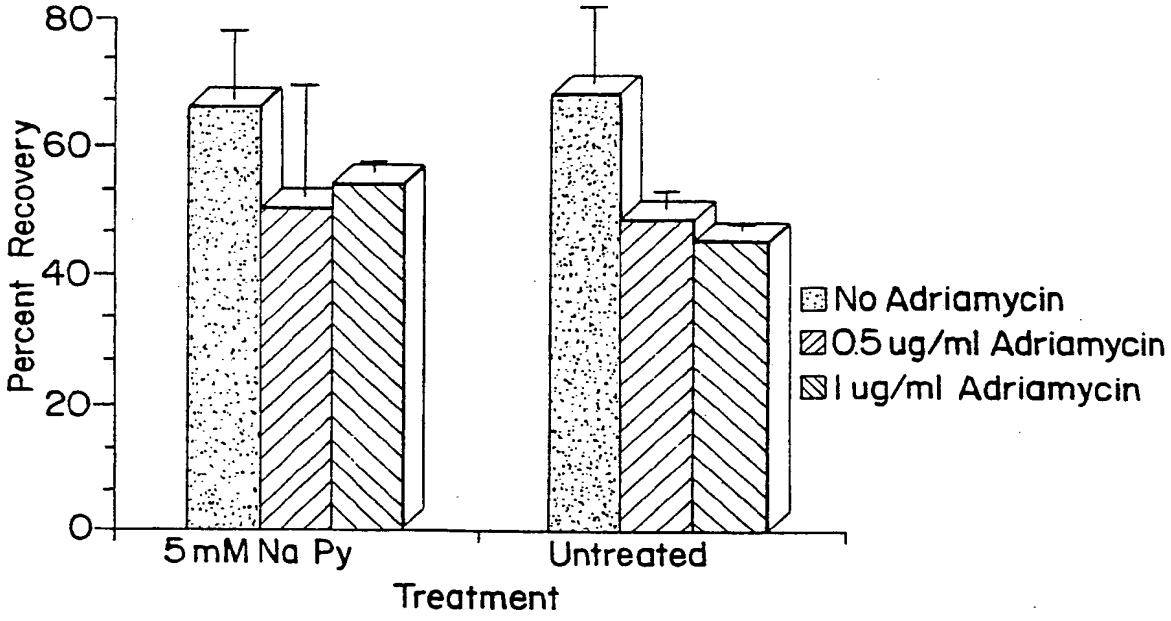


FIG. 15B

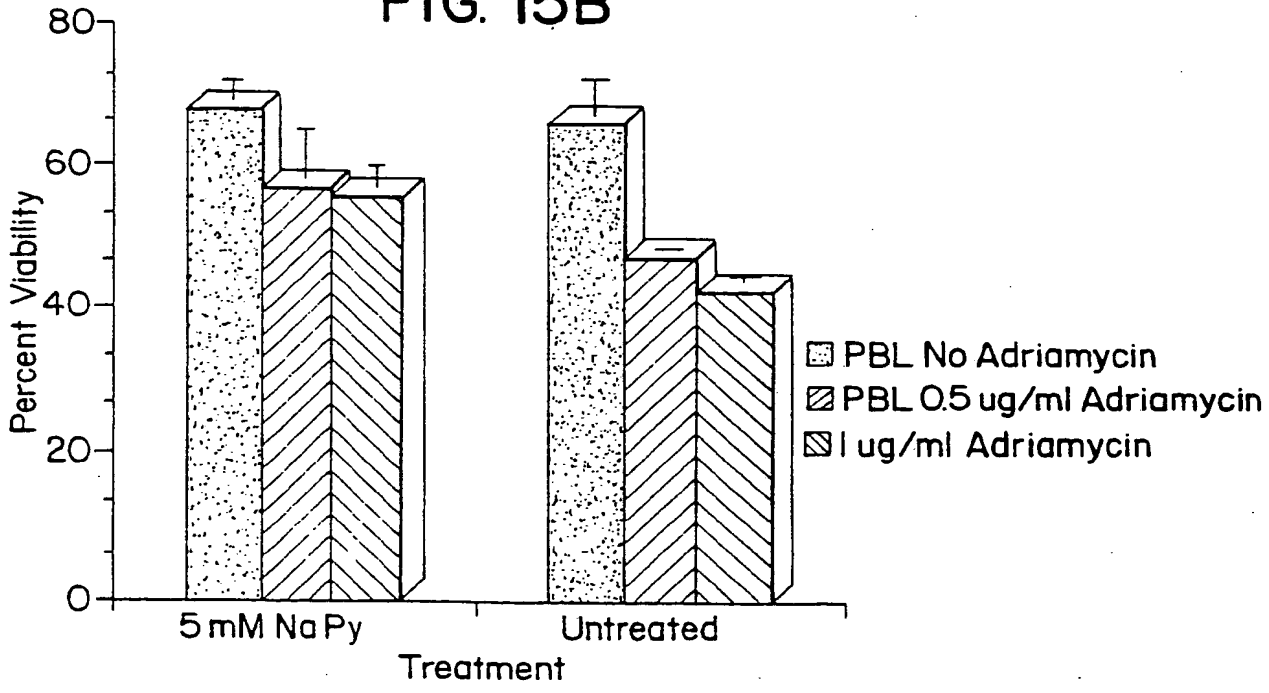


FIG. 16A

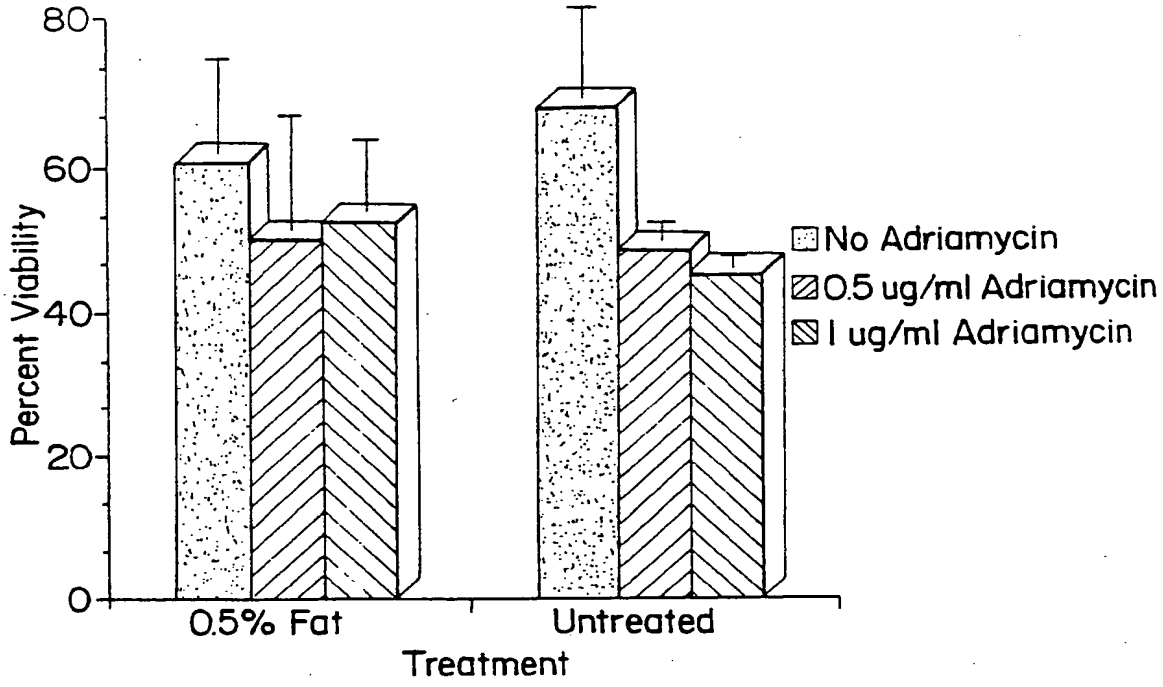


FIG. 16B

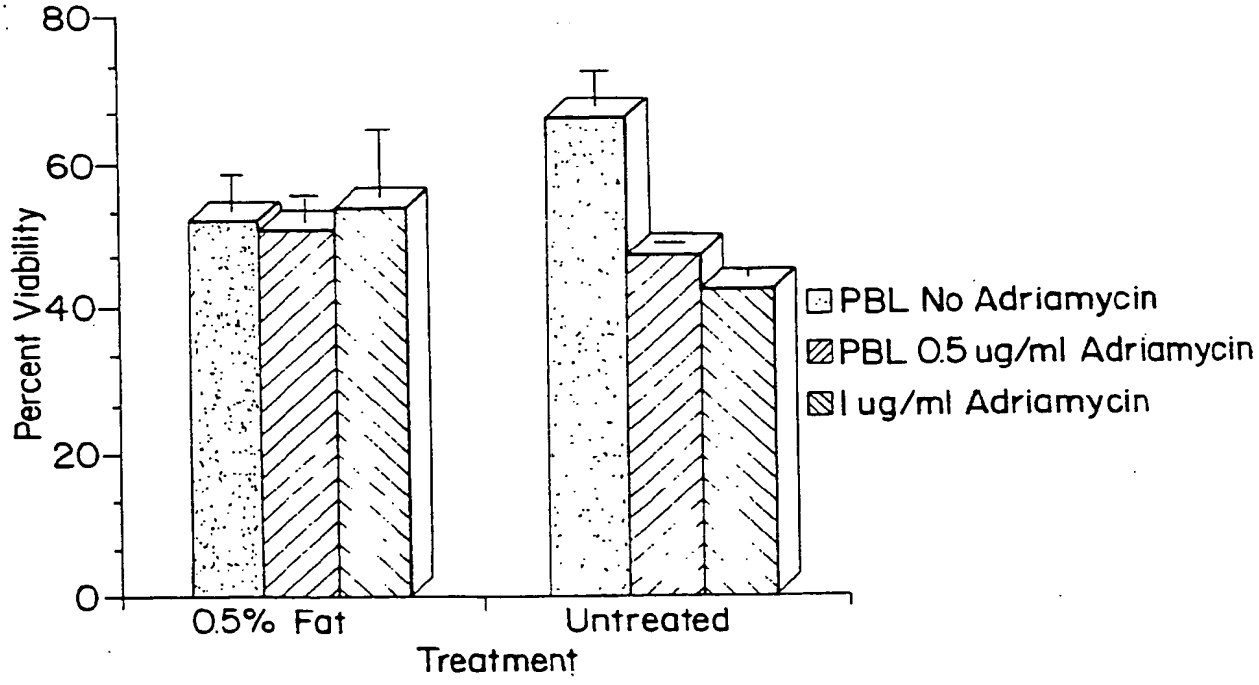


FIG. 17A

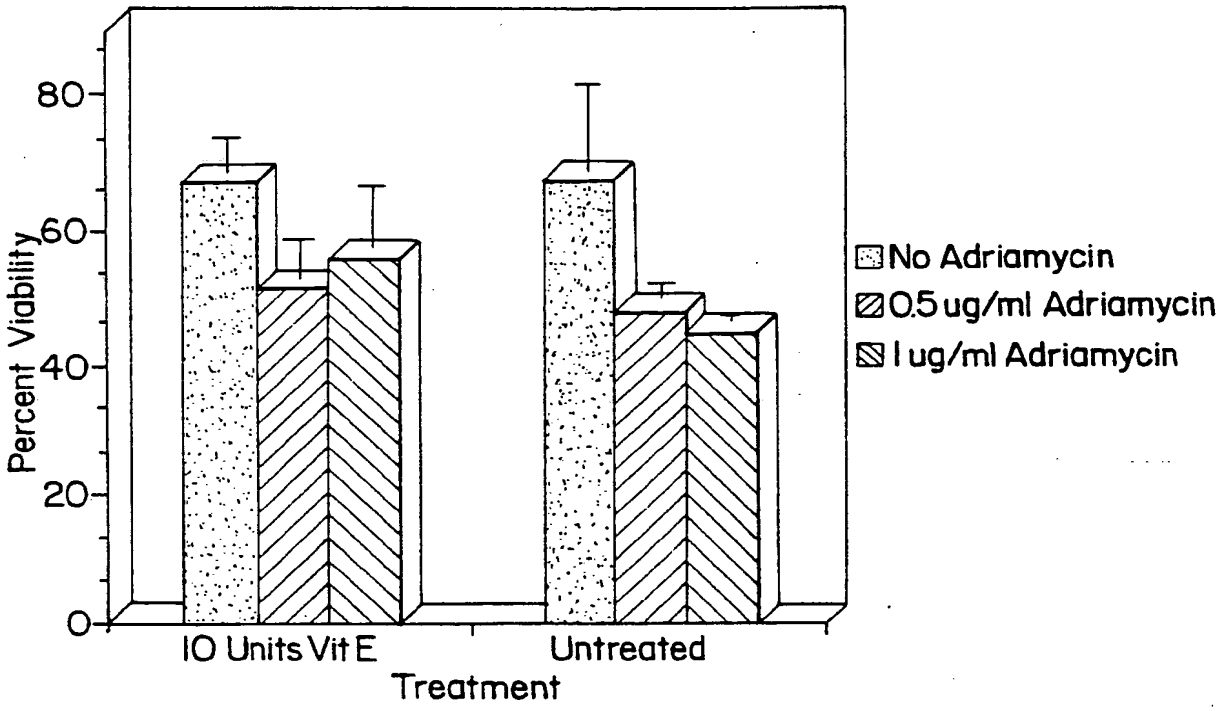


FIG. 17B

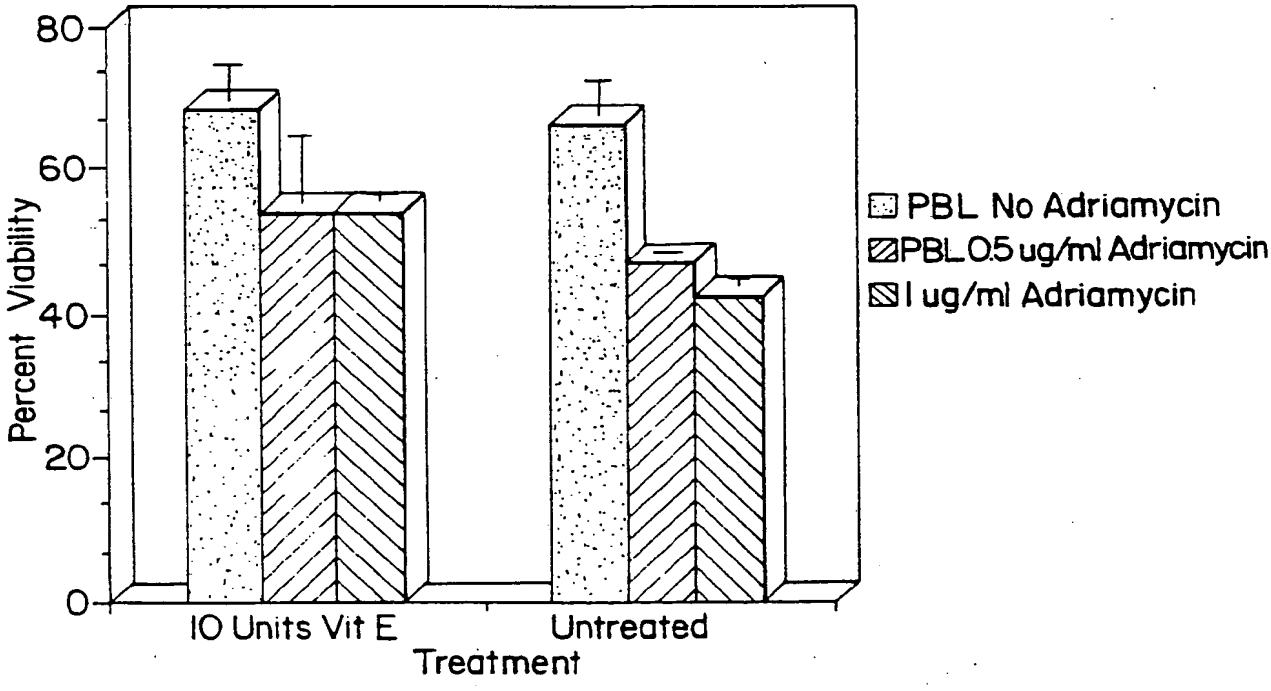


FIG. 18A

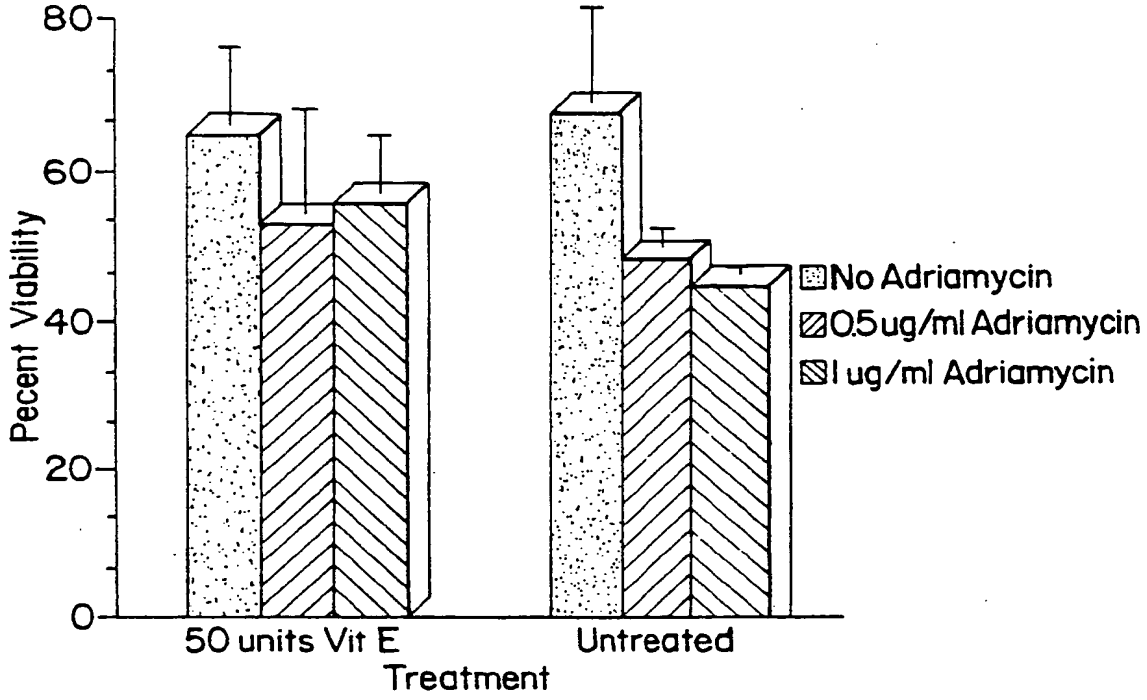


FIG. 18B

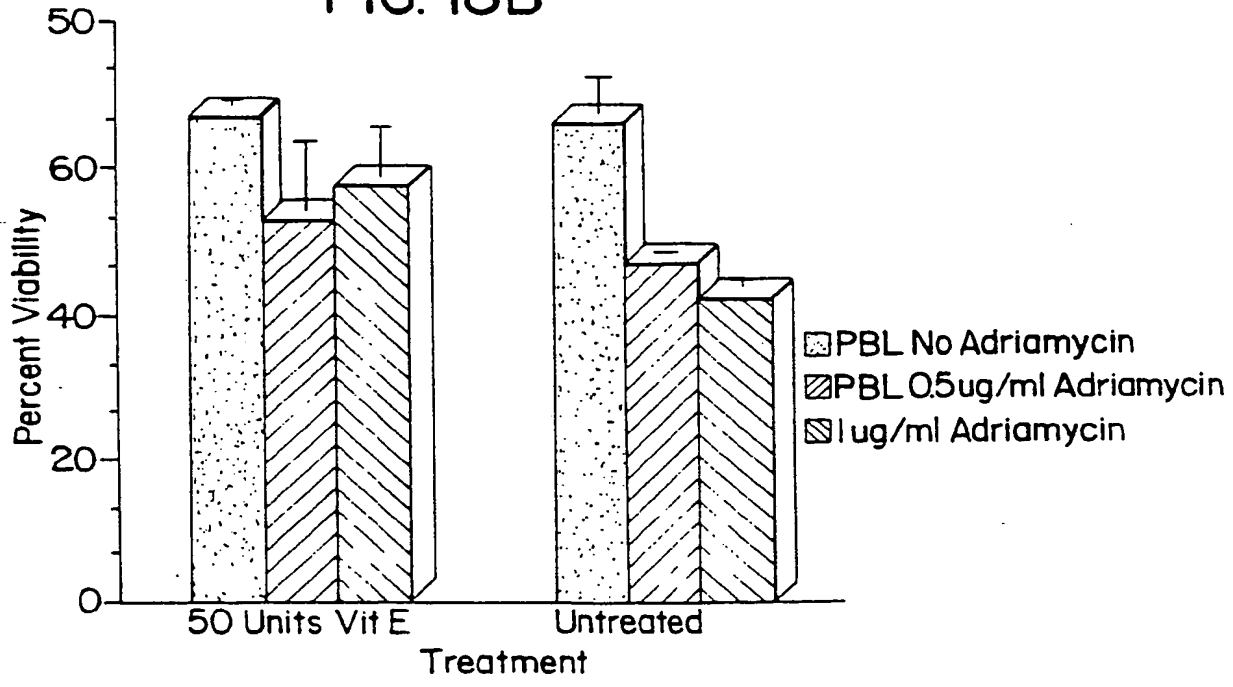


FIG. 19A

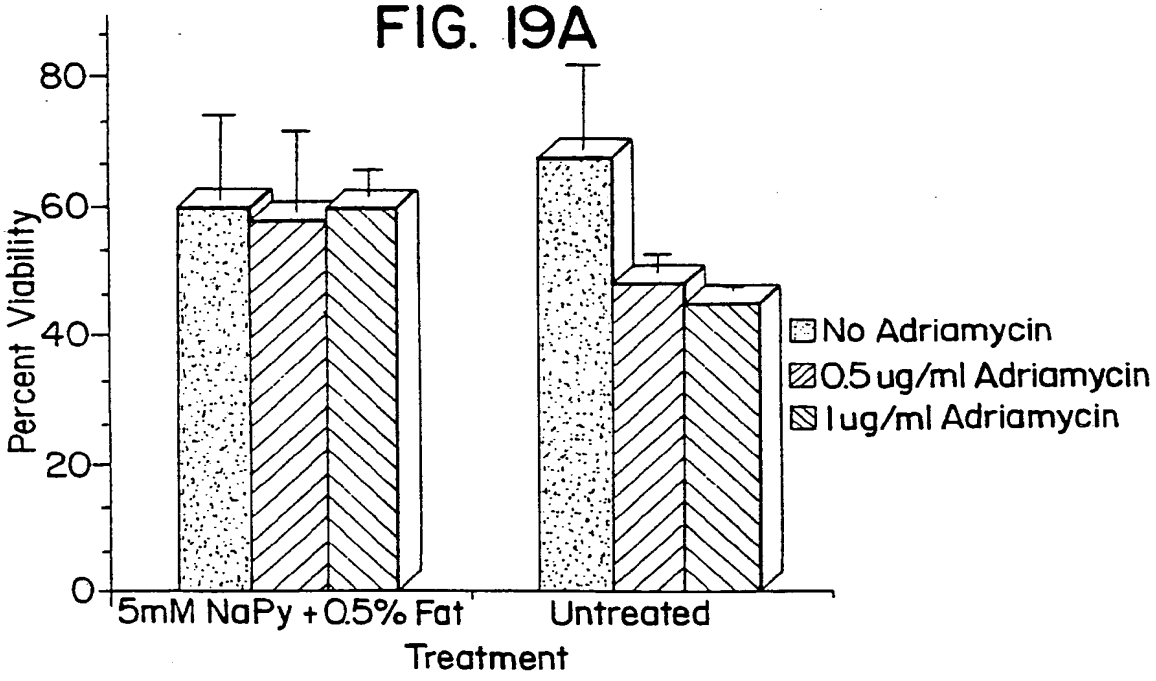


FIG. 19B

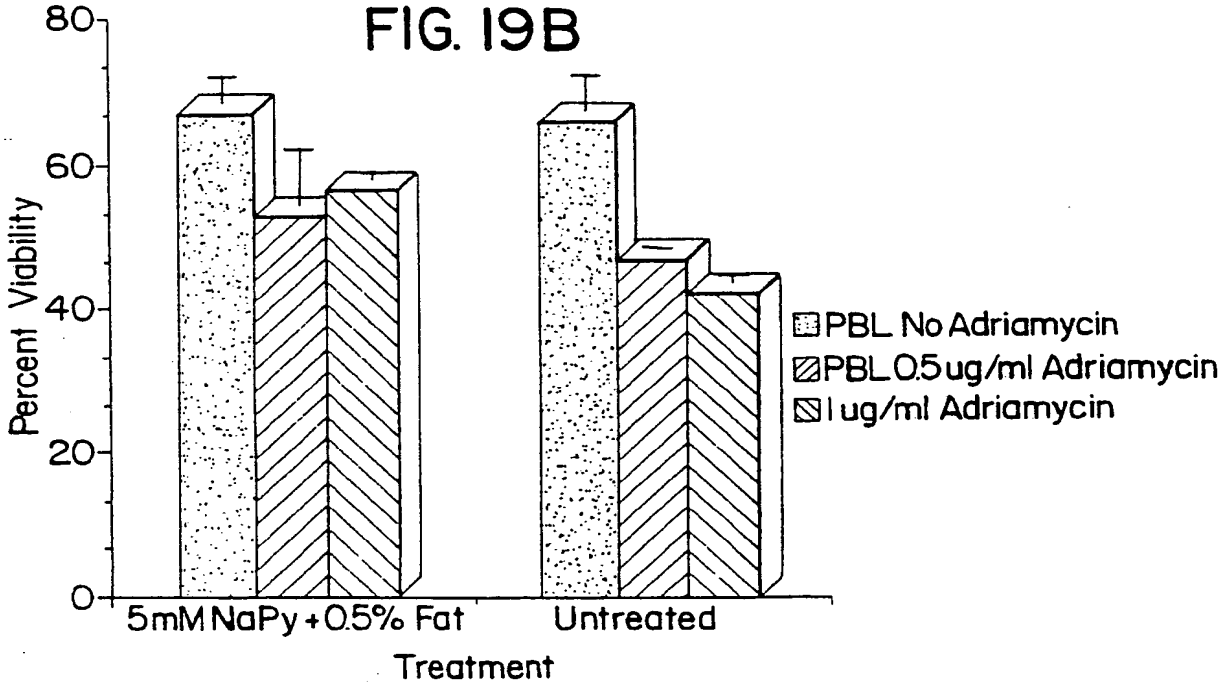


FIG. 20A

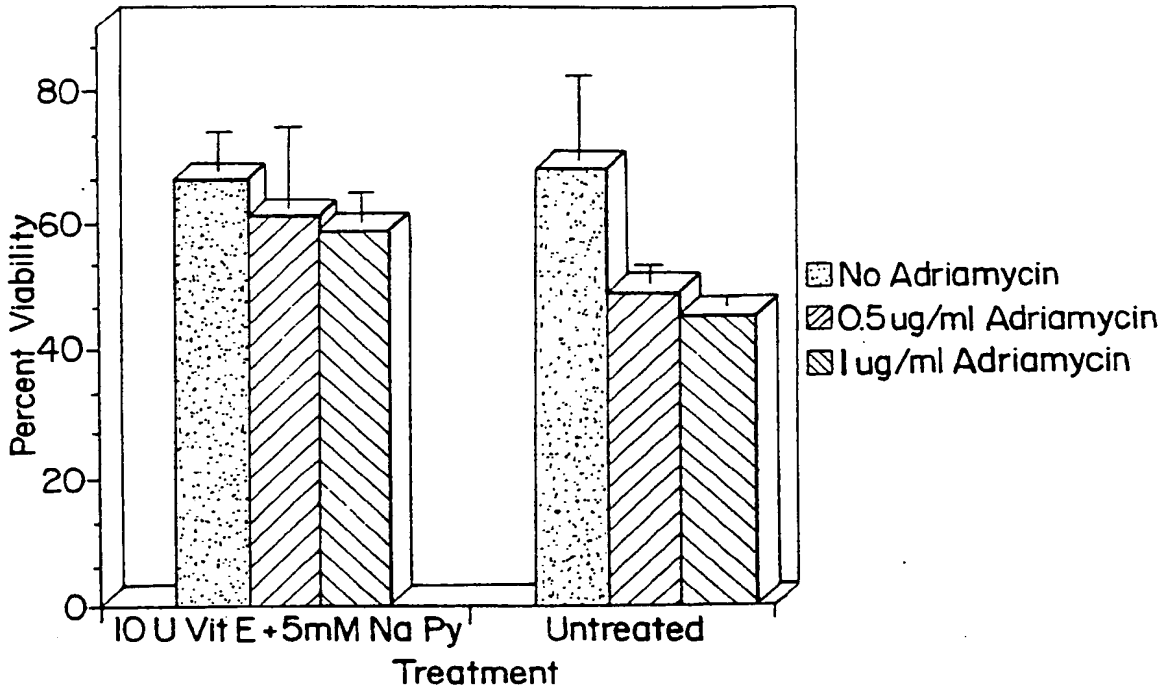


FIG. 20B

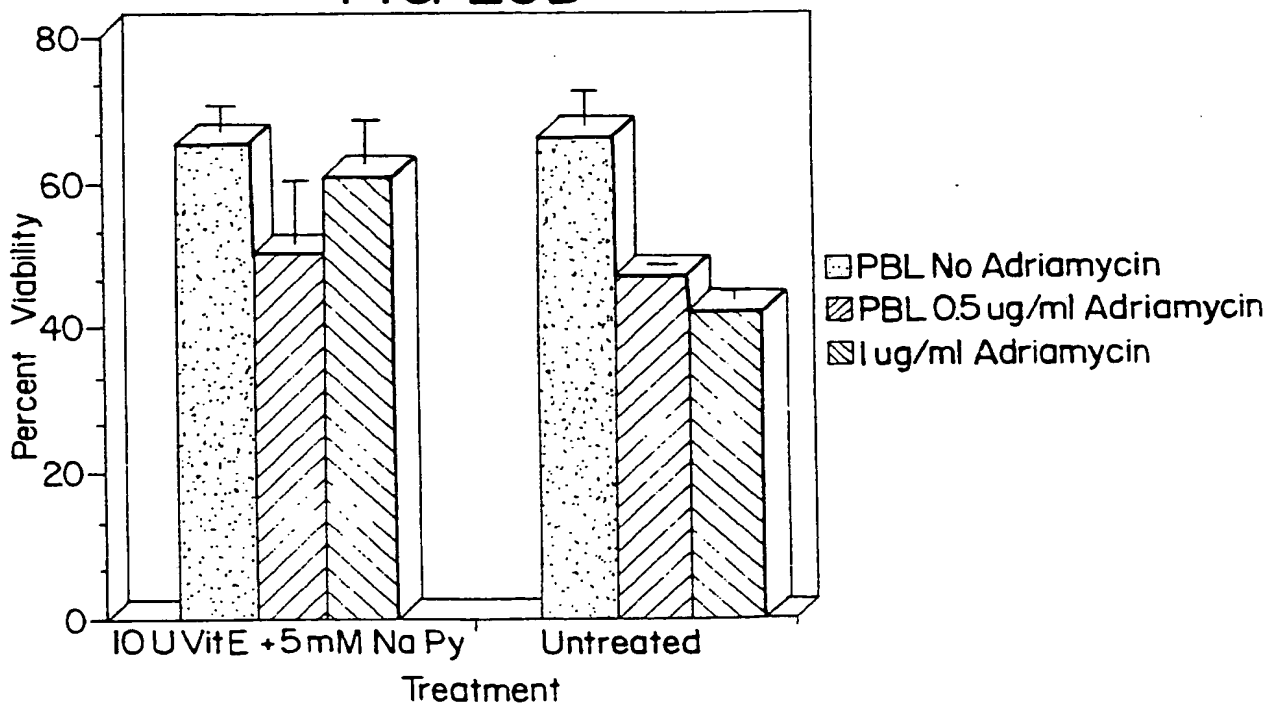


FIG. 21A

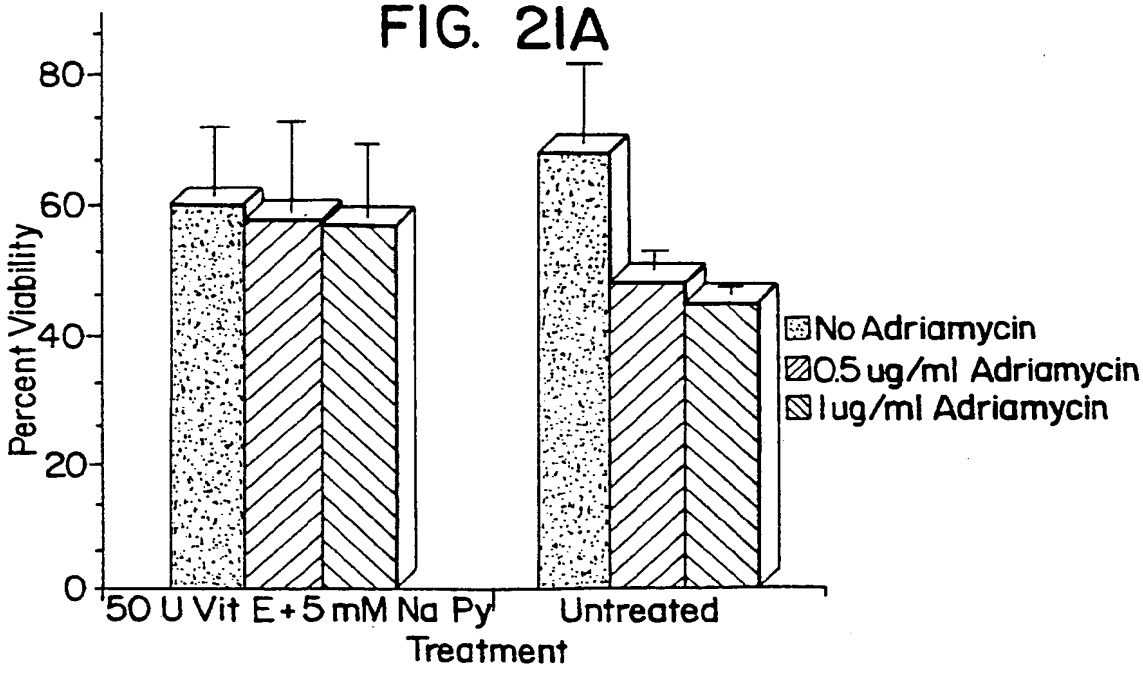


FIG. 21B

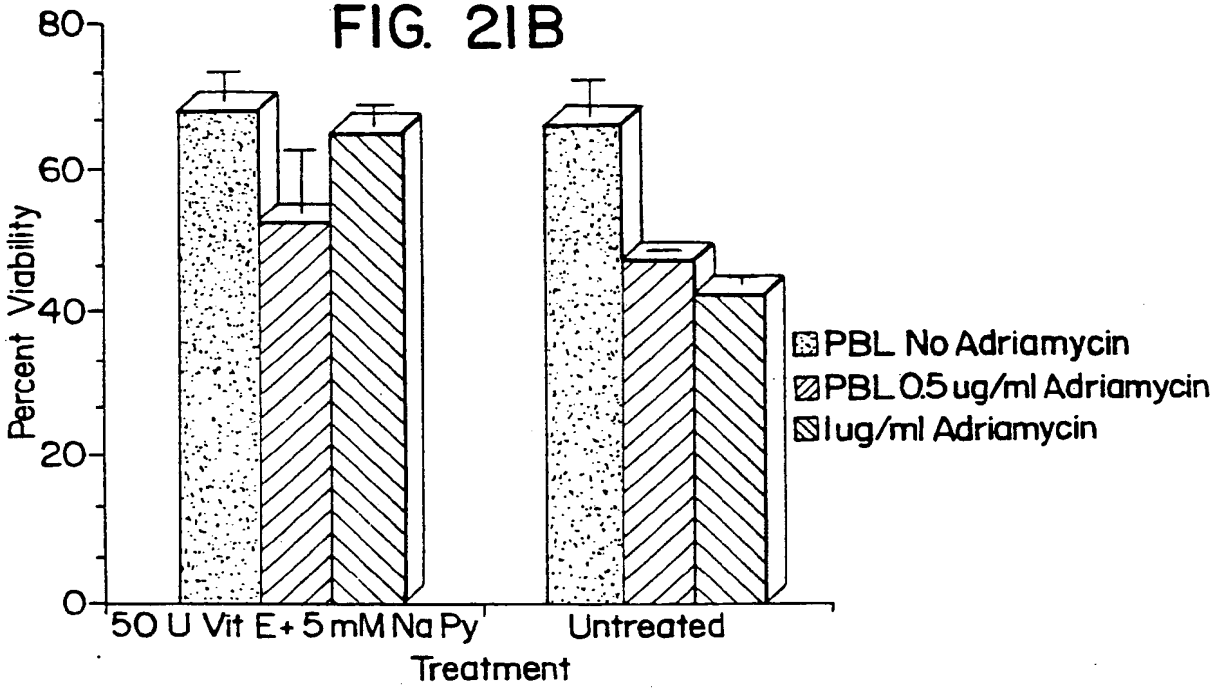


FIG. 22A

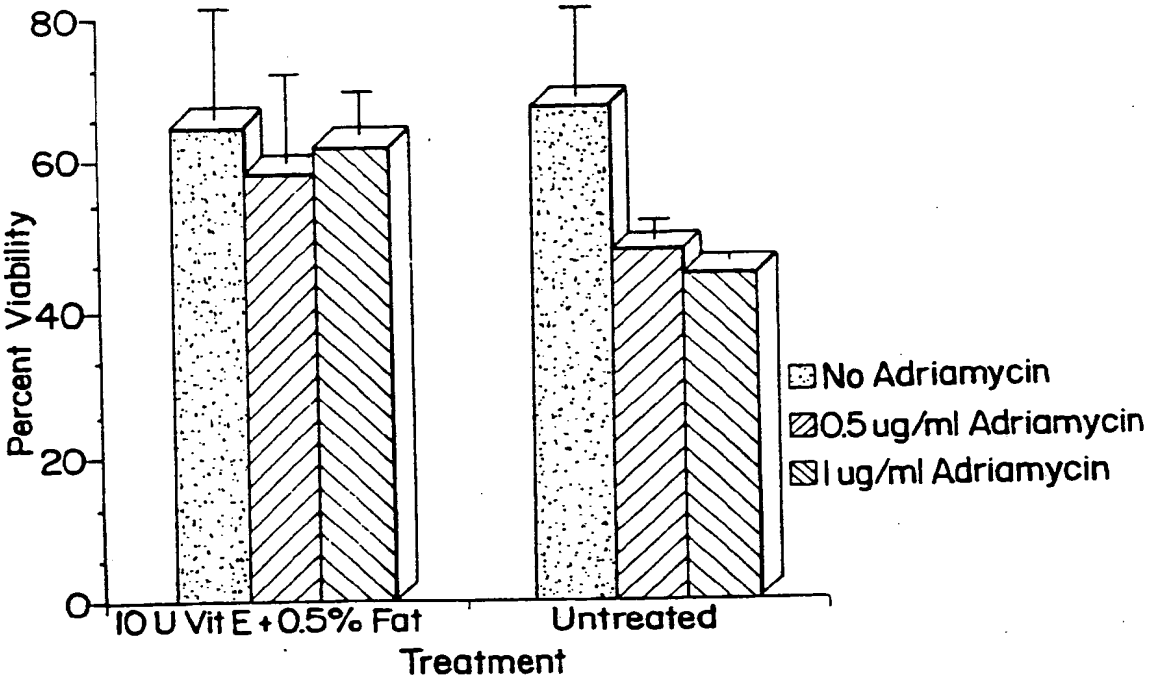


FIG. 22B

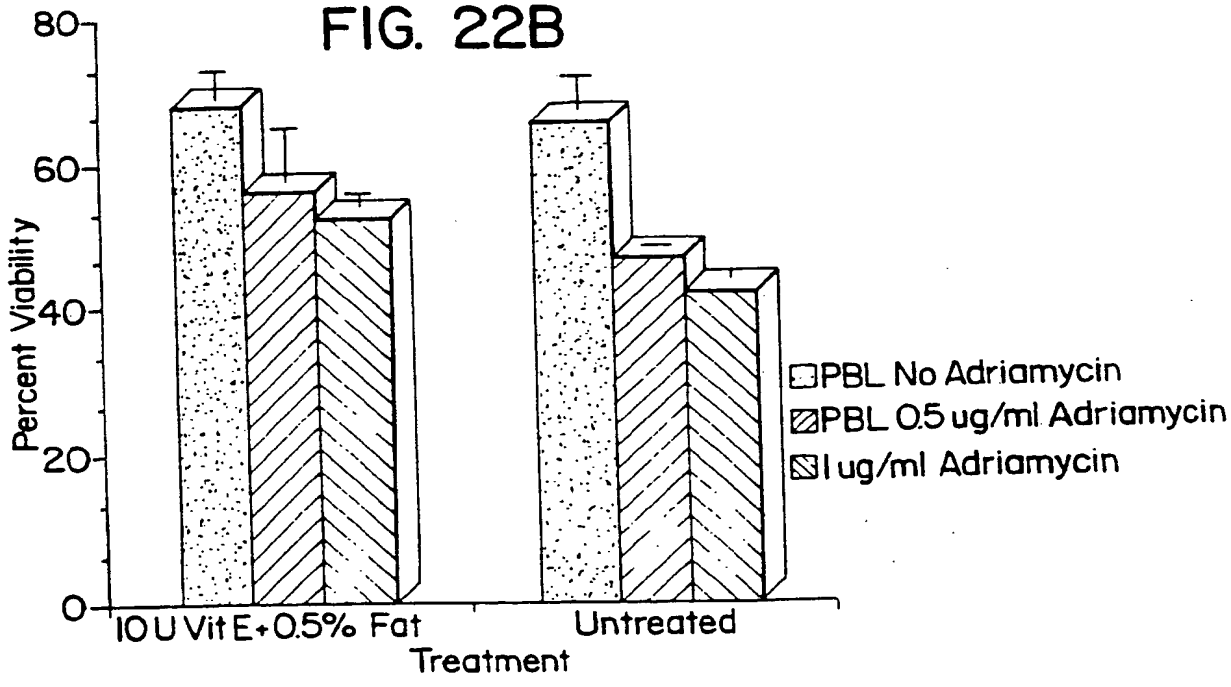


FIG. 23A

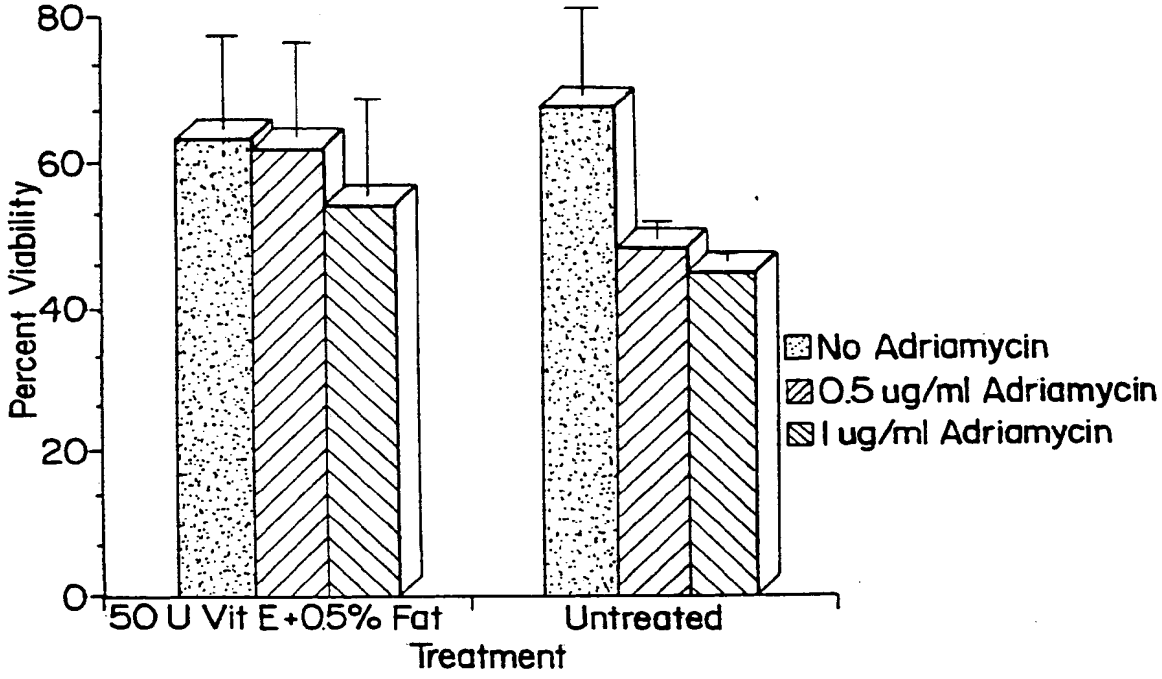


FIG. 23B

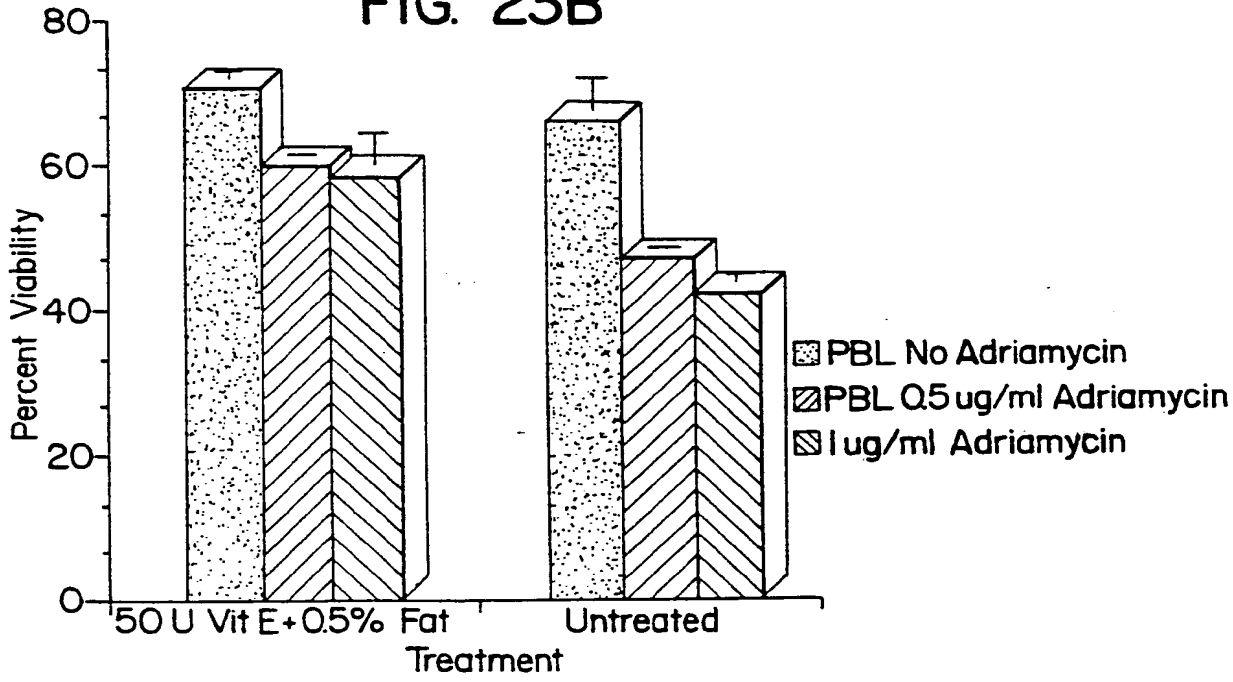


FIG. 24A

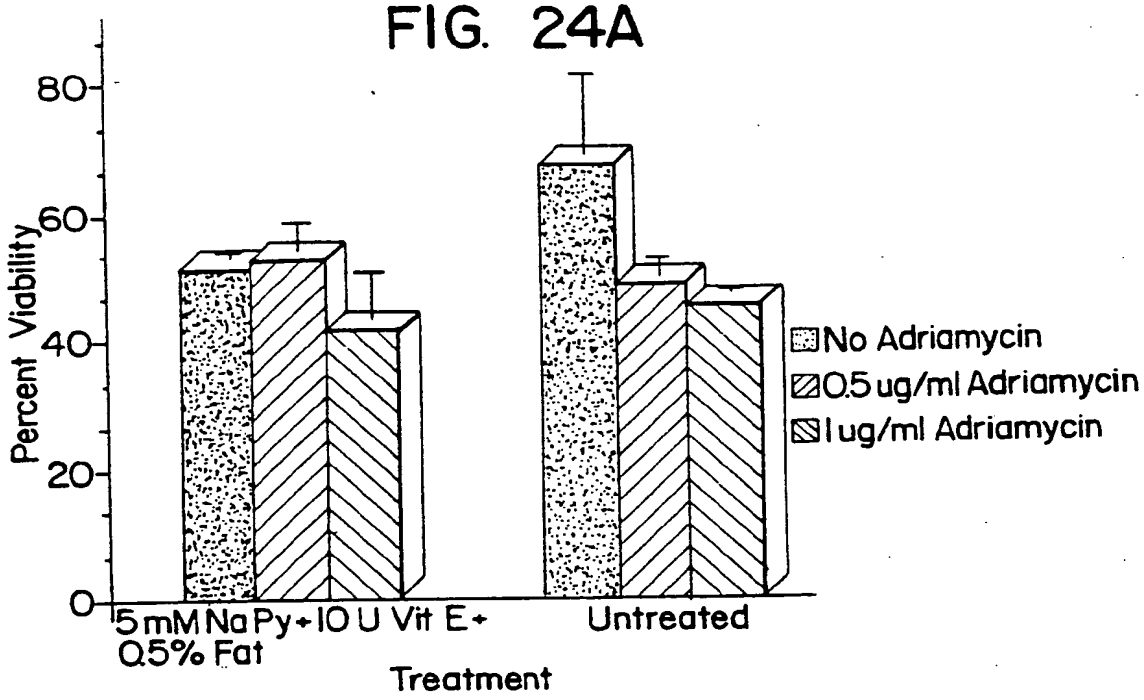


FIG. 24A

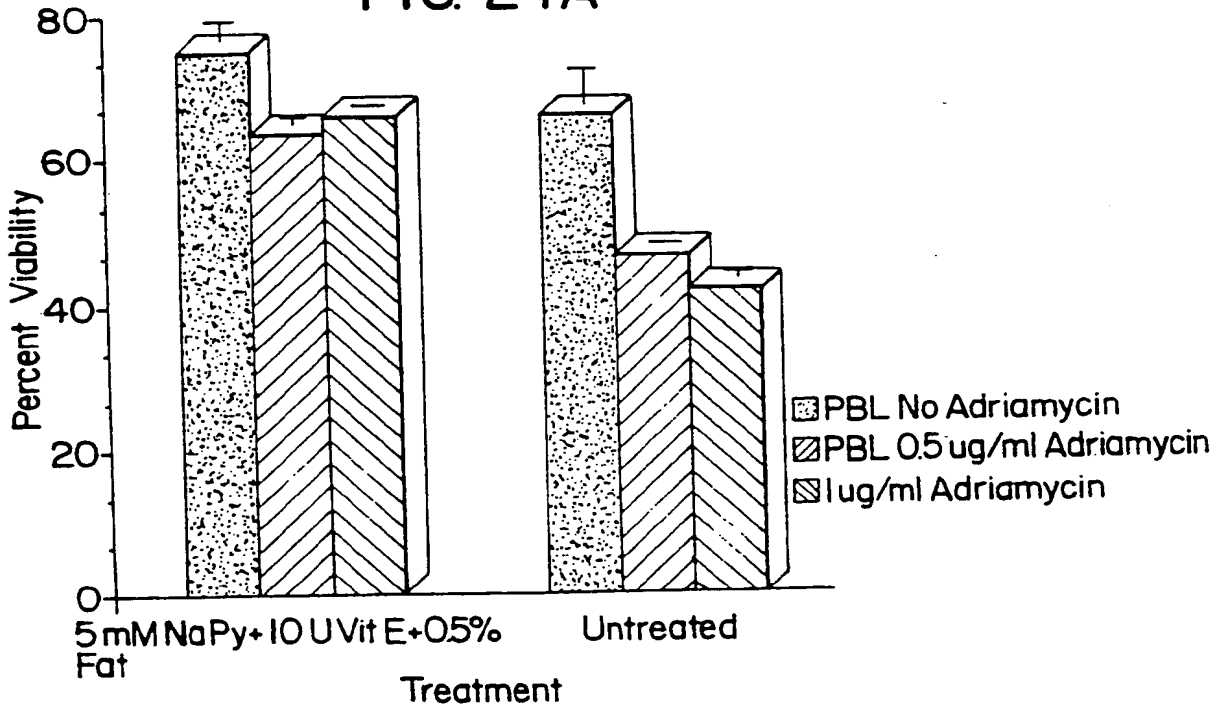


FIG. 25A

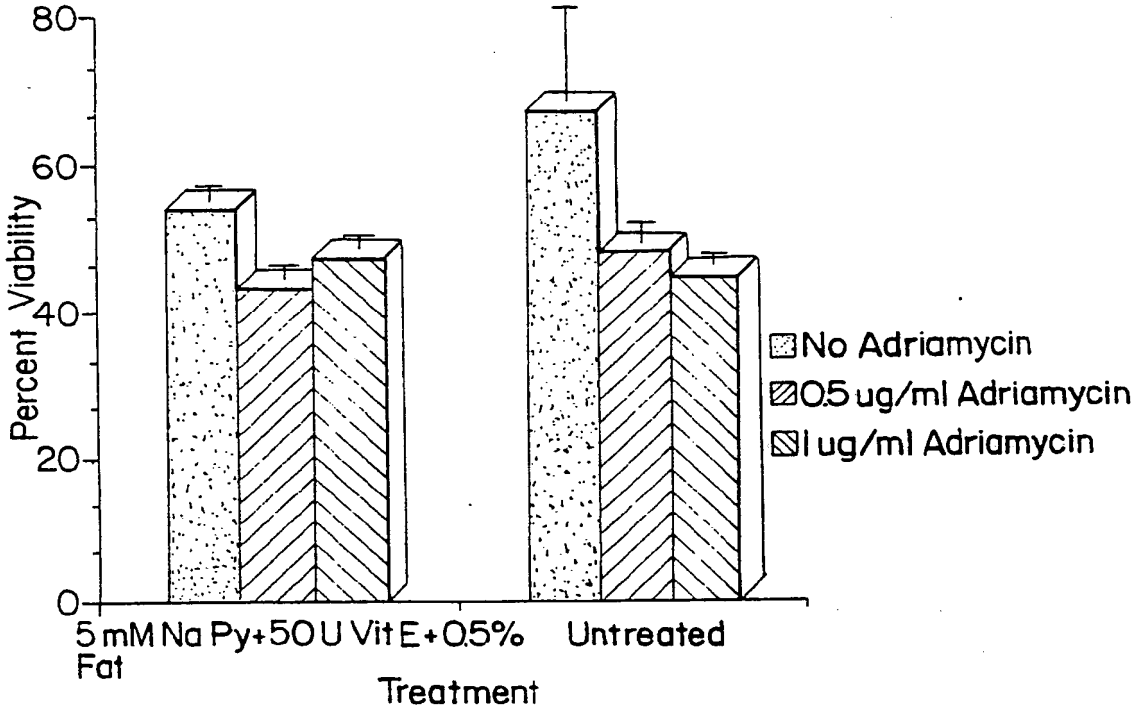
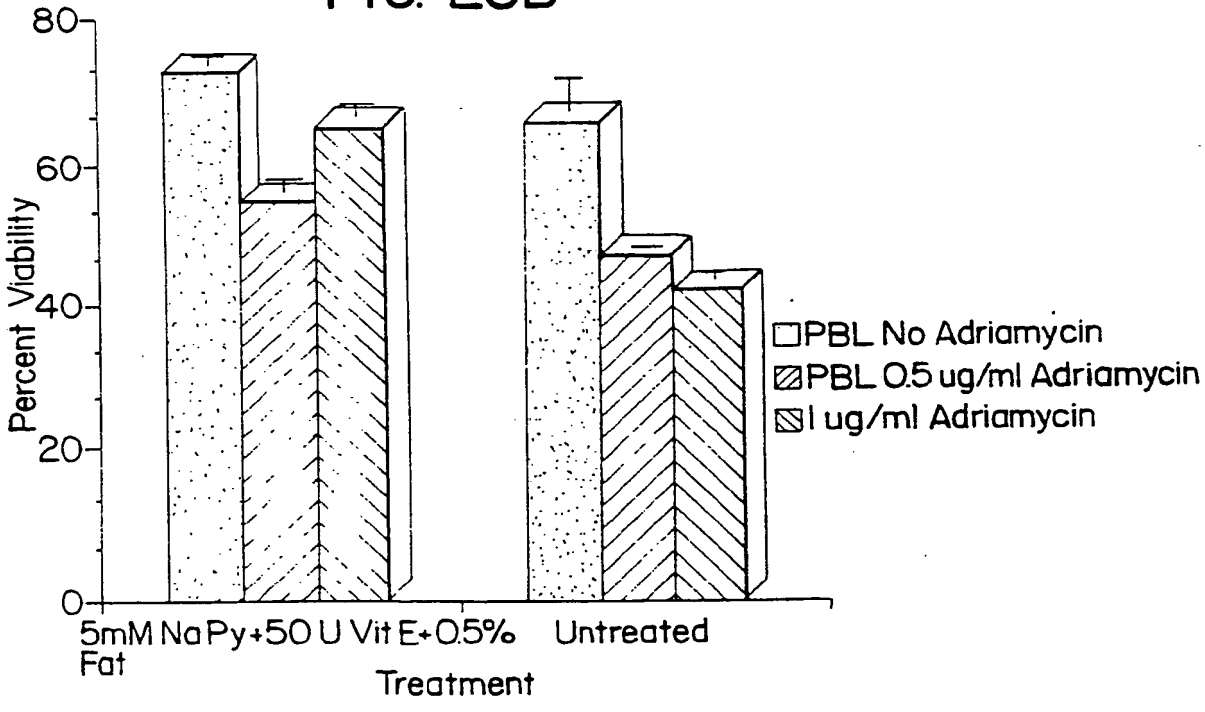


FIG. 25B



INTERNATIONAL SEARCH REPORT

International Application No PCT/US 93/00260

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl. 5 A61K31/19; A61K31/71; //(A61K31/19,31:20, 31:355, 31:375,31:07,31:015)

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
Int.Cl. 5	A61K

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category °	Citation of Document,[11] with indication, where appropriate, of the relevant passages[12]	Relevant to Claim No.[13]
P,X	WO,A,9 215 292 (WARNER-LAMBERT COMPANY) 17 September 1992 see abstract; claims	1-29
E	WO,A,9 310 776 (WARNER-LAMBERT COMPANY) 10 June 1993 see abstract; claims	1-29
X	WO,A,8 700 753 (R.L. LINDSTROM) 12 February 1987 see the whole document & US,A,4 696 917 cited in the application & US,A,4 725 586 cited in the application	1-7,20, 27

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IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
30 JULY 1993	17. 08. 93
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	ORVIZ DIAZ P.

Form PCT/ISA/210 (second sheet) (January 1985)

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category °	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
X	EP,A,0 345 082 (K.K. HAYASHIBARA SEIBUTSU KAGAKU KENKYUJO) 6 December 1989 see the whole document, especially examples 8 and 18 and the claims	1-17,20, 21,27
X	J. ASSOC. OFF. ANAL. CHEM. vol. 74, no. 3, 1991, pages 522 - 525 A. MARTIN 'A resuscitation/selection system for rapid determination of Salmonella in foods.' see the whole document	1-17, 20-21,27
X	DATABASE WPI Section Ch, Week 7618, Derwent Publications Ltd., London, GB; Class B05, AN 76-33105X & JP,A,51 032 738 (M. NAKANISHI) see abstract	1-7,20, 27
Y	CANCER CHEMOTHER. PHARMACOL. vol. 5, no. 3, 1981, pages 175 - 179 M.H. COHEN 'Cure of advanced L1210 leukemia after correction of abnormal red blood cell deformability' cited in the application see the whole document	1-29
Y	IRCS MED. SCI. vol. 7, no. 10, 1979, page 505 S.M. AHMED 'Effects of ascorbic acid and various keto-acids on the glyoxalase enzyme system in mouse and rat liver.' see the whole document	1-29
Y	J. CLIN. INVEST. vol. 88, no. 6, 1991, pages 1886 - 1893 A.K. SALAHUDEEN 'Hydrogen peroxide-induced renal injury. A protective role for pyruvate in vitro and in vivo.' see the whole document	1-29
Y	FREE RADICAL RES. COMMUN. vol. 4, no. 5, 1988, pages 283 - 290 S.D. VARMA 'Peroxide damage to the eye lens in vitro prevention by pyruvate.' see the whole document	1-29

-/--

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category °	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
Y	J. EXP. MED. vol. 165, no. 2, 1987, pages 500 - 514 J. O'DONNELL-TORMEY 'Secretion of pyruvate. An antioxidant defense of mammalian cells.' see the whole document ---	1-29
Y	TOXICOL. LETT. vol. 28, no. 2-3, 1985, page 93-98 U. ANDRAE 'Pyruvate and related alpha-ketoacids protect mammalian cells in culture against hydrogen peroxide-induced cytotoxicity.' see the whole document -----	1-29

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 93/ 00260

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
REMARK: Although claims 24-26 are directed to a method of treatment of the human or animal body the search has been carried out and based on the alleged effects of the compositions.

2. [ ] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO.

US 9300260
SA 73527

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.
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Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A-9215292	17-09-92	AU-A- 1271892	06-10-92
WO-A-9310776	10-06-93	None	
WO-A-8700753	12-02-87	US-A- 4696917	29-09-87
		EP-A,B 0232377	19-08-87
		GB-A,B 2186798	26-08-87
		JP-T- 63500720	17-03-88
		US-A- 4886786	12-12-89
		US-A- 4725586	16-02-88
EP-A-0345082	06-12-89	JP-A- 2193917	31-07-90
		JP-A- 1305025	08-12-89

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For more details about this annex : see Official Journal of the European Patent Office, No. 12/82

CORRECTED VERSION *

PCT

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International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 : A61K 31/19, 31/71 // (A61K 31/19 A61K 31:20, 31:35, 31:375 A61K 31:07, 31:015)	A1	(11) International Publication Number: WO 93/16690
		(43) International Publication Date: 2 September 1993 (02.09.93)

(21) International Application Number: PCT/US93/00260

(22) International Filing Date: 13 January 1993 (13.01.93)

(30) Priority data:
841,342 25 February 1992 (25.02.92) US

(71) Applicant: WARNER-LAMBERT COMPANY [US/US]; 201 Tabor Road, Morris Plains, NJ 07950 (US).

(72) Inventor: MARTIN, Alain ; 31 Country Club Drive, Ringoes, NJ 08551 (US).

(74) Agents: BULLITT, Richard, S. et al.; Warner-Lambert Company, 201 Tabor Road, Morris Plains, NJ 07950 (US).

(81) Designated States: AU, CA, JP, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: CYTOPROTECTIVE COMPOSITIONS CONTAINING PYRUVATE AND ANTIOXIDANTS

(57) Abstract

The present invention pertains to cytoprotective compositions for preventing and reducing injury to mammalian cells from a medicament having cytotoxic properties. In one embodiment, the cytoprotective composition comprises (a) pyruvate selected from the group consisting of pyruvic acid, pharmaceutically acceptable salts of pyruvic acid, and mixtures thereof, and (b) an antioxidant. In a second embodiment, the cytoprotective composition comprises pyruvate selected from the group consisting of pyruvic acid, pharmaceutically acceptable salts of pyruvic acid, and mixtures thereof, (b) an antioxidant, and (c) a mixture of saturated and unsaturated fatty acids wherein the fatty acids are those fatty acids required for the repair of cellular membranes and resuscitation of mammalian cells. The cytoprotective compositions may be incorporated into a wide variety of pharmaceutically acceptable carriers to prepare pharmaceutical products. This invention also relates to methods for preparing and using the cytoprotective compositions and the pharmaceutical products in which the cytoprotective compositions may be used.

* (Referred to in PCT Gazette No. 24/1993, Section II)

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CYTOPROTECTIVE COMPOSITIONS CONTAINING PYRUVATE AND ANTIOXIDANTS

BACKGROUND OF THE INVENTION

This application is a continuation-in-part of copending application serial no. 663,500, filed 1 March 1991.

Field of the Invention

This invention pertains to cytoprotective compositions for preventing and reducing injury to mammalian cells from a medicament having cytotoxic properties, and increasing the resuscitation and proliferation rates of the cells. In a first embodiment, the cytoprotective composition comprises (a) pyruvate selected from the group consisting of pyruvic acid, pharmaceutically acceptable salts of pyruvic acid, and mixtures thereof, and (b) an antioxidant. In a second embodiment, the cytoprotective composition comprises pyruvate selected from the group consisting of pyruvic acid, pharmaceutically acceptable salts of pyruvic acid, and mixtures thereof, (b) an antioxidant, and (c) a mixture of saturated and unsaturated fatty acids wherein

the fatty acids are those fatty acids required for the repair of cellular membranes and resuscitation of mammalian cells. The cytoprotective compositions may be incorporated into a wide variety of pharmaceutically acceptable carriers to prepare pharmaceutical products. This invention also relates to methods for preparing and using the cytoprotective compositions and the pharmaceutical products in which the cytoprotective compositions may be used.

Description of the Background

Cancer is a group of neoplastic diseases affecting different organs and systems in the body. A common feature in all cancers is cellular mutation and abnormal and uncontrolled cell growth usually at a rate greater than that of normal body cells. Neither the etiology of cancer nor the manner in which cancer causes death is completely understood.

Significant advances have been made in the chemotherapeutic treatment of cancer. Most anticancer agents act at specific phases of the cell cycle and are therefore only active against cells in the process of division. Although differences in the duration of the cell cycle occur between different types of cells, all cells show a similar pattern during the division process which may be characterized as follows: (1) a presynthetic phase; (2) a DNA synthesis phase; (3) a postsynthetic phase following termination of DNA synthesis; and (4) a mitosis phase, wherein the cell containing a double complement of DNA divides into two daughter cells. Most anti-neoplastic agents act specifically on processes such as the DNA synthesis phase, the transcription phase, or the mitosis phase and are therefore considered cell-cycle specific agents.

A problem with the chemotherapeutic treatment of cancer is that normal cells which proliferate rapidly, such as those in bone marrow, hair follicles, and the gastrointestinal tract, are often damaged or killed by the anti-neoplastic agents. This cytotoxicity problem occurs because the metabolism of cancer cells is similar to that of normal cells and anticancer agents lack specificity for cancer cells. Because most of the metabolic differences between normal and neoplastic cells are quantitative, anticancer drugs are usually employed at or near the toxic range in order to obtain satisfactory therapeutic effects.

When cells are injured or killed as a result of a cytotoxic agent, a cytoprotective step is desirable to protect the cells from the cytotoxic agent, resuscitate the injured cells, and help produce new cells to replace the dead cells. Injured cells require low levels of oxygen in the initial stages of recovery to suppress oxidative damage and higher levels of oxygen in the later stages of recovery to stimulate cellular viability and proliferation.

Stressed and injured mammalian cells are often exposed to activated oxygen species such as superoxide (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radical ($OH^\cdot$), and singlet oxygen ($^1O_2$). *In vivo*, these reactive oxygen intermediates are generated by cells in response to aerobic metabolism, catabolism of drugs and other xenobiotics, ultraviolet and x-ray radiation, and the respiratory burst of phagocytic cells (such as white blood cells) to kill invading bacteria. Hydrogen peroxide, for example, is produced during respiration of most living organisms especially by stressed and injured cells.

These active oxygen species can injure and kill cells. An important example of such damage is lipid peroxidation which involves the oxidative degradation of

unsaturated lipids. Lipid peroxidation is highly detrimental to membrane structure and function and can cause numerous cytopathological effects. Cells defend against lipid peroxidation by producing radical scavengers such as superoxide dismutase, catalase, and peroxidase. Injured cells have a decreased ability to produce radical scavengers. Excess hydrogen peroxide can react with DNA to cause backbone breakage, produce mutations, and alter and liberate bases. Hydrogen peroxide can also react with pyrimidines to open the 5, 6-double bond, which reaction inhibits the ability of pyrimidines to hydrogen bond to complementary bases, Hallaender et al. (1971). Such oxidative biochemical injury can result in the loss of cellular membrane integrity, reduced enzyme activity, changes in transport kinetics, changes in membrane lipid content, and leakage of potassium ions, amino acids, and other cellular material.

Antioxidants have been shown to inhibit damage associated with active oxygen species. For example, pyruvate and other *alpha*-ketoacids have been reported to react rapidly and stoichiometrically with hydrogen peroxide to protect cells from cytolytic effects, O'Donnell-Tormey et al., J. Exp. Med., 165, pp. 500-514 (1987).

United States patents nos. 3,920,835, 3,984,556, and 3,988,470, all issued to Van Scott et al., disclose methods for treating acne, dandruff, and palmar keratosis, respectively, which consist of applying to the affected area a topical composition comprising from about 1% to about 20% of a lower aliphatic compound containing from two to six carbon atoms selected from the group consisting of *alpha*-hydroxyacids, *alpha*-ketoacids and esters thereof, and 3-hydroxybutryic acid in a pharmaceutically acceptable carrier. The aliphatic compounds include pyruvic acid and lactic acid.

United States patents nos. 4,105,783 and 4,197,316, both issued to Yu et al., disclose a method and composition, respectively, for treating dry skin which consists of applying to the affected area a topical composition comprising from about 1% to about 20% of a compound selected from the group consisting of amides and ammonium salts of *alpha*-hydroxyacids, *beta*-hydroxyacids, and *alpha*-ketoacids in a pharmaceutically acceptable carrier. The compounds include the amides and ammonium salts of pyruvic acid and lactic acid.

United States patent no. 4,234,599, issued to Van Scott et al., discloses a method for treating actinic and nonactinic skin keratoses which consists of applying to the affected area a topical composition comprising an effective amount of a compound selected from the group consisting of *alpha*-hydroxyacids, *beta*-hydroxyacids, and *alpha*-ketoacids in a pharmaceutically acceptable carrier. The acidic compounds include pyruvic acid and lactic acid.

United States patent no. 4,294,852, issued to Wildnauer et al., discloses a composition for treating skin which comprises the *alpha*-hydroxyacids, *beta*-hydroxyacids, and *alpha*-ketoacids disclosed above by Van Scott et al. in combination with C_3-C_8 aliphatic alcohols.

United States patent no. 4,663,166, issued to Veech, discloses an electrolyte solution which comprises a mixture of L-lactate and pyruvate in a ratio from 20:1 to 1:1, respectively, or a mixture of D-*beta*-hydroxybutyrate and acetoacetate, in a ratio from 6:1 to 0.5:1, respectively.

Sodium pyruvate has been reported to reduce the number of erosions, ulcers, and hemorrhages on the gastric mucosa in guinea pigs and rats caused by acetylsalicylic acid. The analgesic and antipyretic

properties of acetylsalicylic acid were not impaired by sodium pyruvate, Puschmann, Arzneimittelforschung, 33, pp. 410-415 and 415-416 (1983).

Pyruvate has been reported to exert a positive inotropic effect in stunned myocardum, which is a prolonged ventricular dysfunction following brief periods of coronary artery occlusions which does not produce irreversible damage, Mentzer et al., Ann. Surg., 209, pp. 629-633 (1989).

Pyruvate has been reported to produce a relative stabilization of left ventricular pressure and work parameter and to reduce the size of infarctions. Pyruvate improves resumption of spontaneous beating of the heart and restoration of normal rates and pressure development, Bunger et al., J. Mol. Cell. Cardiol., 18, pp. 423-438 (1986), Mochizuki et al., J. Physiol. (Paris), 76, pp. 805-812 (1980), Regitz et al., Cardiovasc. Res., 15, pp. 652-658 (1981), Giannelli et al., Ann. Thorac. Surg., 21, pp. 386-396 (1976).

Sodium pyruvate has been reported to act as an antagonist to cyanide intoxification (presumably through the formation of a cyanohydrin) and to protect against the lethal effects of sodium sulfide and to retard the onset and development of functional, morphological, and biochemical measures of acrylamide neuropathy of axons, Schwartz et al., Toxicol. Appl. Pharmacol., 50, pp. 437-442 (1979), Sabri et al., Brain Res., 483, pp. 1-11 (1989).

A chemotherapeutic cure of advanced L1210 leukemia has been reported using sodium pyruvate to restore abnormally deformed red blood cells to normal. The deformed red blood cells prevented adequate drug delivery to tumor cells, Cohen, Cancer Chemother. Pharmacol., 5, pp. 175-179 (1981).

Primary cultures of heterotopic tracheal transplant exposed *in vivo* to 7, 12-dimethyl-benz(a)anthracene were reported to be successfully maintained in enrichment medium supplemented with sodium pyruvate along with cultures of interleukin-2 stimulated peripheral blood lymphocytes, and plasmacytomas and hybridomas, pig embryos, and human blastocysts, Shacter, J. Immunol. Methods, 99, pp. 259-270 (1987), Marchok et al., Cancer Res., 37, pp. 1811-1821 (1977), Davis, J. Reprod. Fertil. Suppl., 33, pp. 115-124 (1985), Okamoto et al., No To Shinkei, 38, pp. 593-598 (1986), Cohen et al., J. In Vitro Fert. Embryo Transfer, 2, pp. 59-64 (1985).

United States patents nos. 4,158,057, 4,351,835, 4,415,576, and 4,645,764, all issued to Stanko, disclose methods for preventing the accumulation of fat in the liver of a mammal due to the ingestion of alcohol, for controlling weight in a mammal, for inhibiting body fat while increasing protein concentration in a mammal, and for controlling the deposition of body fat in a living being, respectively. The methods comprise administering to the mammal a therapeutic mixture of pyruvate and dihydroxyacetone, and optionally riboflavin. United States patent no. 4,548,937, issued to Stanko, discloses a method for controlling the weight gain of a mammal which comprises administering to the mammal a therapeutically effective amount of pyruvate, and optionally riboflavin. United States patent no. 4,812,479, issued to Stanko, discloses a method for controlling the weight gain of a mammal which comprises administering to the mammal a therapeutically effective amount of dihydroxyacetone, and optionally riboflavin and pyruvate.

Rats fed a calcium-oxalate lithogenic diet including sodium pyruvate were reported to develop fewer urinary calculi (stones) than control rats not given

sodium pyruvate, Ogawa et al., Hinyokika Kiyo, 32, pp. 1341-1347 (1986).

United States patent no. 4,521,375, issued to Houlsby, discloses a method for sterilizing surfaces which come into contact with living tissue. The method comprises sterilizing the surface with aqueous hydrogen peroxide and then neutralizing the surface with pyruvic acid.

United States patent no. 4,416,982, issued to Tauda et al., discloses a method for decomposing hydrogen peroxide by reacting the hydrogen peroxide with a phenol or aniline derivative in the presence of peroxidase.

United States patent no. 4,696,917, issued to Lindstrom et al., discloses an eye irrigation solution which comprises Eagle's Minimum Essential Medium with Earle's salts, chondroitin sulfate, a buffer solution, 2-mercaptoethanol, and a pyruvate. The irrigation solution may optionally contain ascorbic acid and *alpha*-tocopherol. United States patent no. 4,725,586, issued to Lindstrom et al., discloses an irrigation solution which comprises a balanced salt solution, chondroitin sulfate, a buffer solution, 2-mercaptoethanol, sodium bicarbonate or dextrose, a pyruvate, a sodium phosphate buffer system, and cystine. The irrigation solution may optionally contain ascorbic acid and *gamma*-tocopherol.

United States patent no. 3,887,702 issued to Baldwin, discloses a composition for treating fingernails and toenails which consists essentially of soybean oil or sunflower oil in combination with Vitamin E.

United States patent no. 4,847,069, issued to Bissett et al., discloses a photoprotective composition comprising (a) a sorbohydroxamic acid, (b) an anti-inflammatory agent selected from steroidal anti-inflammatory agents and a natural anti-inflammatory

agent, and (c) a topical carrier. Fatty acids may be present as an emollient. United States patent no. 4,847,071, issued to Bissett et al., discloses a photoprotective composition comprising (a) a tocopherol or tocopherol ester radical scavenger, (b) an anti-inflammatory agent selected from steroidal anti-inflammatory agents and a natural anti-inflammatory agent, and (c) a topical carrier. United States patent no. 4,847,072, issued to Bissett et al., discloses a topical composition comprising not more than 25% tocopherol sorbate in a topical carrier.

United States patent no. 4,533,637, issued to Yamane et al., discloses a culture medium which comprises a carbon source, a nucleic acid source precursor, amino acids, vitamins, minerals, a lipophilic nutrient, and serum albumin, and cyclodextrins. The lipophilic substances include unsaturated fatty acids and lipophilic vitamins such as Vitamin A, D, and E. Ascorbic acid may also be present.

United Kingdom patent application no. 2,196,348A, to Kovar et al., discloses a synthetic culture medium which comprises inorganic salts, monosaccharides, amino acids, vitamins, buffering agents, and optionally sodium pyruvate adding magnesium hydroxide or magnesium oxide to the emulsion. The oil phase may include chicken fat.

United States patent no. 4,284,630, issued to Yu et al., discloses a method for stabilizing a water-in-oil emulsion which comprises adding magnesium hydroxide or magnesium oxide to the emulsion. The oil phase may include chicken fat.

Preparation-H™ has been reported to increase the rate of wound healing in artificially created rectal ulcers. The active ingredients in Preparation-H™ are skin respiratory factor and shark liver oil,

Subramanyam et al., Digestive Diseases and Sciences, 29, pp. 829-832 (1984).

The addition of sodium pyruvate to bacterial and yeast systems has been reported to inhibit hydrogen peroxide production, enhance growth, and protect the systems against the toxicity of reactive oxygen intermediates. The unsaturated fatty acids and saturated fatty acids contained within chicken fat enhanced membrane repair and reduced cytotoxicity. The antioxidants glutathione and thioglycollate reduced the injury induced by oxygen radical species, Martin, Ph.D. thesis, (1987-89).

United States patent no. 4,615,697, issued to Robinson, discloses a controlled release treatment composition comprising a treating agent and a bioadhesive agent comprising a water-swellable but water-insoluble, fibrous cross-linked carboxy-functional polymer.

European patent application no. 0410696A1, to Kellaway et al., discloses a mucoadhesive delivery system comprising a treating agent and a polyacrylic acid cross-linked with from about 1% to about 20% by weight of a polyhydroxy compound such as a sugar, cyclitol, or lower polyhydric alcohol.

While the above therapeutic compositions are reported to inhibit the production of reactive oxygen intermediates, none of the above compositions are entirely satisfactory cytoprotective compositions. None of the compositions has the ability to simultaneously decrease cellular levels of hydrogen peroxide production, increase cellular resistance to cytotoxic agents, increase rates of cellular proliferation, and increase cellular viability to protect and resuscitate mammalian cells. The present invention provides such improved therapeutic cytoprotective compositions without the

disadvantages characteristic of previously known compositions.

SUMMARY OF THE INVENTION

The present invention pertains to cytoprotective compositions for preventing and reducing injury to mammalian cells from a medicament having cytotoxic properties. In a first embodiment, the cytoprotective composition comprises (a) pyruvate selected from the group consisting of pyruvic acid, pharmaceutically acceptable salts of pyruvic acid, and mixtures thereof, and (b) an antioxidant. In a second embodiment, the cytoprotective composition comprises (a) pyruvate selected from the group consisting of pyruvic acid, pharmaceutically acceptable salts of pyruvic acid, and mixtures thereof, (b) an antioxidant, and (c) a mixture of saturated and unsaturated fatty acids wherein the fatty acids are those fatty acids required for the repair of cellular membranes and resuscitation of mammalian cells.

The cytoprotective compositions of the present invention may be administered to cells concurrently with a cytotoxic agent. The cytoprotective compositions may also be administered to cells prior to administration of a cytotoxic anticancer agent to selectively protect non-cancerous cells in the presence of cancerous cells.

The cytoprotective compositions may be incorporated into a wide variety of pharmaceutically acceptable carriers to prepare pharmaceutical products. This invention also relates to methods for preparing and using the cytoprotective compositions and the pharmaceutical products in which the cytoprotective compositions may be used.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1, top portion, is a graph showing the viability of U937 monocytic leukemia tumor cells after 24 hours, as determined by tritiated thymidine incorporation assay, following treatment of the cells with different dosage levels of Doxorubicin. Figure 1, bottom portion, is a graph showing the viability of U937 monocytic leukemia tumor cells after 24 hours, as determined by exclusion of the vital dye trypan blue assay, following treatment of the cells with different dosage levels of Doxorubicin.

Figure 2 is a graph showing the viability of U937 monocytic leukemia tumor cells after 1 hour, as determined by exclusion of the vital dye trypan blue assay, following treatment of the cells with different dosage levels of Doxorubicin.

Figure 3 is a graph showing the viability of U937 monocytic leukemia tumor cells after 24 hours, as determined by tritiated thymidine incorporation assay, following treatment of the cells with the cytoprotective components of the present invention, alone and in combinations, at different dosage levels.

Figure 4, top portion, is a graph showing the viability of U937 monocytic leukemia tumor cells in a wash-out study, as determined by tritiated thymidine incorporation assay, after 24 hour pretreatment of the cells with 5 mM sodium pyruvate followed by administration of different dosage levels of Doxorubicin. Figure 4, bottom portion, is a graph showing the viability of peripheral blood monocytes in a wash-out study, as determined by tritiated thymidine incorporation assay, after 24 hour pretreatment of the cells with 5 mM

sodium pyruvate followed by administration of different dosage levels of Doxorubicin.

Figure 5, top portion, is a graph showing the viability of U937 monocytic leukemia tumor cells in a wash-out study, as determined by tritiated thymidine incorporation assay, after 24 hour pretreatment of the cells with 0.5% fatty acids followed by administration of different dosage levels of Doxorubicin. Figure 5, bottom portion, is a graph showing the viability of peripheral blood monocytes in a wash-out study, as determined by tritiated thymidine incorporation assay, after 24 hour pretreatment of the cells with 0.5% fatty acids followed by administration of different dosage levels of Doxorubicin.

Figure 6, top portion, is a graph showing the viability of U937 monocytic leukemia tumor cells in a wash-out study, as determined by tritiated thymidine incorporation assay, after 24 hour pretreatment of the cells with 10 U Vitamin E followed by administration of different dosage levels of Doxorubicin. Figure 6, bottom portion, is a graph showing the viability of peripheral blood monocytes in a wash-out study, as determined by tritiated thymidine incorporation assay, after 24 hour pretreatment of the cells with 10 U Vitamin E followed by administration of different dosage levels of Doxorubicin.

Figure 7, top portion, is a graph showing the viability of U937 monocytic leukemia tumor cells in a wash-out study, as determined by tritiated thymidine incorporation assay, after 24 hour pretreatment of the cells with 50 U Vitamin E followed by administration of different dosage levels of Doxorubicin. Figure 7, bottom portion, is a graph showing the viability of peripheral blood monocytes in a wash-out study, as determined by tritiated thymidine incorporation assay, after 24 hour pretreatment of the cells with 50 U Vitamin E followed by administration of different dosage levels of Doxorubicin.

Figure 8, top portion, is a graph showing the viability of U937 monocytic leukemia tumor cells in a wash-out study, as determined by tritiated thymidine incorporation assay, after 24 hour pretreatment of the cells with 5 mM sodium pyruvate and 0.5% fatty acids followed by administration of different dosage levels of Doxorubicin. Figure 8, bottom portion, is a graph showing the viability of peripheral blood monocytes in a wash-out study, as determined by tritiated thymidine incorporation assay, after 24 hour pretreatment of the cells with 5 mM sodium pyruvate and 0.5% fatty acids followed by administration of different dosage levels of Doxorubicin.

Figure 9, top portion, is a graph showing the viability of U937 monocytic leukemia tumor cells in a wash-out study, as determined by tritiated thymidine incorporation assay, after 24 hour pretreatment of the cells with 5 mM sodium pyruvate and 10 U Vitamin E followed by administration of different dosage levels of Doxorubicin. Figure 9, bottom portion, is a graph showing the viability of peripheral blood monocytes in a wash-out study, as determined by tritiated thymidine incorporation assay, after 24 hour pretreatment of the cells with 5 mM sodium pyruvate and 10 U Vitamin E followed by administration of different dosage levels of Doxorubicin.

Figure 10, top portion, is a graph showing the viability of U937 monocytic leukemia tumor cells in a wash-out study, as determined by tritiated thymidine incorporation assay, after 24 hour pretreatment of the cells with 5 mM sodium pyruvate and 50 U Vitamin E followed by administration of different dosage levels of Doxorubicin. Figure 10, bottom portion, is a graph showing the viability of peripheral blood monocytes in a wash-out study, as determined by tritiated thymidine incorporation assay, after 24 hour pretreatment of the

cells with 5 mM sodium pyruvate and 50 U Vitamin E followed by administration of different dosage levels of Doxorubicin.

Figure 11, top portion, is a graph showing the viability of U937 monocytic leukemia tumor cells in a wash-out study, as determined by tritiated thymidine incorporation assay, after 24 hour pretreatment of the cells with 10 U Vitamin E and 0.5% fatty acids followed by administration of different dosage levels of Doxorubicin. Figure 11, bottom portion, is a graph showing the viability of peripheral blood monocytes in a wash-out study, as determined by tritiated thymidine incorporation assay, after 24 hour pretreatment of the cells with 10 U Vitamin E and 0.5% fatty acids followed by administration of different dosage levels of Doxorubicin.

Figure 12, top portion, is a graph showing the viability of U937 monocytic leukemia tumor cells in a wash-out study, as determined by tritiated thymidine incorporation assay, after 24 hour pretreatment of the cells with 50 U Vitamin E and 0.5% fatty acids followed by administration of different dosage levels of Doxorubicin. Figure 12, bottom portion, is a graph showing the viability of peripheral blood monocytes in a wash-out study, as determined by tritiated thymidine incorporation assay, after 24 hour pretreatment of the cells with 50 U Vitamin E and 0.5% fatty acids followed by administration of different dosage levels of Doxorubicin.

Figure 13, top portion, is a graph showing the viability of U937 monocytic leukemia tumor cells in a wash-out study, as determined by tritiated thymidine incorporation assay, after 24 hour pretreatment of the cells with 5 mM sodium pyruvate, 10 U Vitamin E, and 0.5% fatty acids followed by administration of different dosage levels of Doxorubicin. Figure 13, bottom portion,

is a graph showing the viability of peripheral blood monocytes in a wash-out study, as determined by tritiated thymidine incorporation assay, after 24 hour pretreatment of the cells with 5 mM sodium pyruvate, 10 U Vitamin E, and 0.5% fatty acids followed by administration of different dosage levels of Doxorubicin.

Figure 14, top portion, is a graph showing the viability of U937 monocytic leukemia tumor cells in a wash-out study, as determined by tritiated thymidine incorporation assay, after 24 hour pretreatment of the cells with 5 mM sodium pyruvate, 50 U Vitamin E, and 0.5% fatty acids followed by administration of different dosage levels of Doxorubicin. Figure 14, bottom portion, is a graph showing the viability of peripheral blood monocytes in a wash-out study, as determined by tritiated thymidine incorporation assay, after 24 hour pretreatment of the cells with 5 mM sodium pyruvate, 50 U Vitamin E, and 0.5% fatty acids followed by administration of different dosage levels of Doxorubicin.

Figure 15, top portion, is a graph showing the viability of U937 monocytic leukemia tumor cells in a co-culture study, as determined by tritiated thymidine incorporation assay, after 24 hour pretreatment of the cells with 5 mM sodium pyruvate followed by administration of different dosage levels of Doxorubicin. Figure 15, bottom portion, is a graph showing the viability of peripheral blood monocytes in a co-culture study, as determined by tritiated thymidine incorporation assay, after 24 hour pretreatment of the cells with 5 mM sodium pyruvate followed by administration of different dosage levels of Doxorubicin.

Figure 16, top portion, is a graph showing the viability of U937 monocytic leukemia tumor cells in a co-culture study, as determined by tritiated thymidine incorporation assay, after 24 hour pretreatment of the cells with 0.5% fatty acids followed by administration of

different dosage levels of Doxorubicin. Figure 16, bottom portion, is a graph showing the viability of peripheral blood monocytes in a co-culture study, as determined by tritiated thymidine incorporation assay, after 24 hour pretreatment of the cells with 0.5% fatty acids followed by administration of different dosage levels of Doxorubicin.

Figure 17, top portion, is a graph showing the viability of U937 monocytic leukemia tumor cells in a co-culture study, as determined by tritiated thymidine incorporation assay, after 24 hour pretreatment of the cells with 10 U Vitamin E followed by administration of different dosage levels of Doxorubicin. Figure 17, bottom portion, is a graph showing the viability of peripheral blood monocytes in a co-culture study, as determined by tritiated thymidine incorporation assay, after 24 hour pretreatment of the cells with 10 U Vitamin E followed by administration of different dosage levels of Doxorubicin.

Figure 18, top portion, is a graph showing the viability of U937 monocytic leukemia tumor cells in a co-culture study, as determined by tritiated thymidine incorporation assay, after 24 hour pretreatment of the cells with 50 U Vitamin E followed by administration of different dosage levels of Doxorubicin. Figure 18, bottom portion, is a graph showing the viability of peripheral blood monocytes in a co-culture study, as determined by tritiated thymidine incorporation assay, after 24 hour pretreatment of the cells with 50 U Vitamin E followed by administration of different dosage levels of Doxorubicin.

Figure 19, top portion, is a graph showing the viability of U937 monocytic leukemia tumor cells in a co-culture study, as determined by tritiated thymidine incorporation assay, after 24 hour pretreatment of the cells with 5 mM sodium pyruvate and 0.5% fatty acids

followed by administration of different dosage levels of Doxorubicin. Figure 19, bottom portion, is a graph showing the viability of peripheral blood monocytes in a co-culture study, as determined by tritiated thymidine incorporation assay, after 24 hour pretreatment of the cells with 5 mM sodium pyruvate and 0.5% fatty acids followed by administration of different dosage levels of Doxorubicin.

Figure 20, top portion, is a graph showing the viability of U937 monocytic leukemia tumor cells in a co-culture study, as determined by tritiated thymidine incorporation assay, after 24 hour pretreatment of the cells with 5 mM sodium pyruvate and 10 U Vitamin E followed by administration of different dosage levels of Doxorubicin. Figure 20, bottom portion, is a graph showing the viability of peripheral blood monocytes in a co-culture study, as determined by tritiated thymidine incorporation assay, after 24 hour pretreatment of the cells with 5 mM sodium pyruvate and 10 U Vitamin E followed by administration of different dosage levels of Doxorubicin.

Figure 21, top portion, is a graph showing the viability of U937 monocytic leukemia tumor cells in a co-culture study, as determined by tritiated thymidine incorporation assay, after 24 hour pretreatment of the cells with 5 mM sodium pyruvate and 50 U Vitamin E followed by administration of different dosage levels of Doxorubicin. Figure 21, bottom portion, is a graph showing the viability of peripheral blood monocytes in a co-culture study, as determined by tritiated thymidine incorporation assay, after 24 hour pretreatment of the cells with 5 mM sodium pyruvate and 50 U Vitamin E followed by administration of different dosage levels of Doxorubicin.

Figure 22, top portion, is a graph showing the viability of U937 monocytic leukemia tumor cells in a co-

culture study, as determined by tritiated thymidine incorporation assay, after 24 hour pretreatment of the cells with 10 U Vitamin E and 0.5% fatty acids followed by administration of different dosage levels of Doxorubicin. Figure 22, bottom portion, is a graph showing the viability of peripheral blood monocytes in a co-culture study, as determined by tritiated thymidine incorporation assay, after 24 hour pretreatment of the cells with 10 U Vitamin E and 0.5% fatty acids followed by administration of different dosage levels of Doxorubicin.

Figure 23, top portion, is a graph showing the viability of U937 monocytic leukemia tumor cells in a co-culture study, as determined by tritiated thymidine incorporation assay, after 24 hour pretreatment of the cells with 50 U Vitamin E and 0.5% fatty acids followed by administration of different dosage levels of Doxorubicin. Figure 23, bottom portion, is a graph showing the viability of peripheral blood monocytes in a co-culture study, as determined by tritiated thymidine incorporation assay, after 24 hour pretreatment of the cells with 50 U Vitamin E and 0.5% fatty acids followed by administration of different dosage levels of Doxorubicin.

Figure 24, top portion, is a graph showing the viability of U937 monocytic leukemia tumor cells in a co-culture study, as determined by tritiated thymidine incorporation assay, after 24 hour pretreatment of the cells with 5 mM sodium pyruvate, 10 U Vitamin E, and 0.5% fatty acids followed by administration of different dosage levels of Doxorubicin. Figure 24, bottom portion, is a graph showing the viability of peripheral blood monocytes in a co-culture study, as determined by tritiated thymidine incorporation assay, after 24 hour pretreatment of the cells with 5 mM sodium pyruvate, 10 U Vitamin E, and 0.5% fatty acids followed by administration of different dosage levels of Doxorubicin.

Figure 25, top portion, is a graph showing the viability of U937 monocytic leukemia tumor cells in a co-culture study, as determined by tritiated thymidine incorporation assay, after 24 hour pretreatment of the cells with 5 mM sodium pyruvate, 50 U Vitamin E, and 0.5% fatty acids followed by administration of different dosage levels of Doxorubicin. Figure 25, bottom portion, is a graph showing the viability of peripheral blood monocytes in a co-culture study, as determined by tritiated thymidine incorporation assay, after 24 hour pretreatment of the cells with 5 mM sodium pyruvate, 50 U Vitamin E, and 0.5% fatty acids followed by administration of different dosage levels of Doxorubicin.

DETAILED DESCRIPTION OF THE INVENTION

Applicant has discovered cytoprotective compositions for protecting mammalian cells from a medicament having cytotoxic properties by preventing and reducing injury to the cells. Cells treated with the cytoprotective compositions of the present invention show decreased levels of hydrogen peroxide production, increased resistance to cytotoxic agents, increased rates of proliferation, and increased viability. The cytoprotective compositions may be administered to cells concurrently with a cytotoxic agent or the cytoprotective compositions may be administered to cells prior to administration of a cytotoxic anticancer agent to selectively protect non-cancerous cells in the presence of cancerous cells. Because cancerous cells have a rapid metabolism, cancerous cells will rapidly consume the protective cytoprotective composition and will not be protected by the cytoprotective compositions when the chemotherapeutic medicament is subsequently administered.

The term "injured cell" as used herein means a cell which has (a) injured membranes so that transport through the membranes is diminished resulting in an increase in toxins and normal cellular wastes inside the cell and a decrease in nutrients and other components necessary for cellular repair inside the cell, (b) an increase in concentration of oxygen radicals inside the cell because of the decreased ability of the cell to produce antioxidants and enzymes, and (c) damaged DNA, RNA, and ribosomes which must be repaired or replaced before normal cellular functions can be resumed. The term "resuscitation" of injured mammalian cells as used herein means the reversal of cytotoxicity, the stabilization of the cellular membrane, an increase in the proliferation rate of the cell, and/or the normalization of cellular functions such as the secretion of growth factors, hormones, and the like. The term "cytotoxicity" as used herein means a condition caused by a cytotoxic agent that injures the cell. Injured cells do not proliferate because injured cells expend all energy on cellular repair. Aiding cellular repair promotes cellular proliferation.

Epidermal keratinocytic cells and monocytic cells have multiple oxygen generating mechanisms and the degree to which each type of mechanism functions differs in each type of cell. In monocytes, for example, the respiratory bursting process is more pronounced than in epidermal keratinocytes. Hence, the components in the cytoprotective compositions of the present invention may vary depending upon the types of cells involved in the condition being treated.

In a first embodiment, the therapeutic cytoprotective composition for treating mammalian cells, preferably epidermal keratinocytes, comprises (a) pyruvate, (b) an antioxidant, and (c) a mixture of saturated and unsaturated fatty acids.

While not wishing to be bound by theory, applicant believes that pyruvate (or pyruvic acid) can be transported inside a cell where it can act as an antioxidant to neutralize oxygen radicals in the cell. Pyruvate can also be used inside the cell in the citric acid cycle to provide energy to increase cellular viability, and as a precursor in the synthesis of important biomolecules to promote cellular proliferation. In addition, pyruvate can be used in the multifunction oxidase system to reverse cytotoxicity. Antioxidants, especially lipid-soluble antioxidants, can be absorbed into the cell membrane to neutralize oxygen radicals and thereby protect the membrane. The combination of pyruvate inside the cell and an antioxidant in the cellular membrane functions in a synergistic manner to reduce hydrogen peroxide production in the cell to levels lower than can be achieved by use of either type of component alone.

The saturated and unsaturated fatty acids in the present invention are those fatty acids required for the repair of cellular membranes and resuscitation of mammalian cells. Hence, the fatty acids in the cytoprotective composition, which may be in the form of mono-, di-, and/or triglycerides or free fatty acids, are readily available for the repair of injured cells and the production of new cells to replace dead cells. Cells injured by oxygen radicals need to produce unsaturated fatty acids to repair cellular membranes. However, the production of unsaturated fatty acids by cells requires oxygen. Thus, the injured cell needs high levels of oxygen to produce unsaturated fatty acids and at the same time needs to reduce the level of oxygen within the cell to reduce oxidative injury. By providing the cell with the unsaturated fatty acids needed for repair, the need of the cell to produce unsaturated fatty acids is reduced and the need for high oxygen levels is also reduced. The presence of mixtures of saturated and unsaturated fatty acids in the cytoprotective composition significantly

enhances the ability of pyruvate and the antioxidant to inhibit reactive oxygen production. By stabilizing the cellular membrane, unsaturated fatty acids also improve membrane function and enhance pyruvate transport into the cell. By improving the viability of the cells, unsaturated fatty acids also improve the repair of cellular membranes rate of the cells. Hence, the three components in the cytoprotective composition function together in a synergistic manner to prevent and reduce injury to mammalian cells, increase the resuscitation rate of injured cells, and increase the production of new cells.

In a second embodiment, the therapeutic cytoprotective composition for treating mammalian cells, preferably epidermal keratinocytes, comprises (a) pyruvate, (b) lactate, and (c) a mixture of saturated and unsaturated fatty acids. In this embodiment, lactate is employed instead of an antioxidant. Antioxidants react with, and neutralize, oxygen radicals after the radicals are already formed. Lactate, on the other hand, is a component in the cellular feedback mechanism and inhibits the respiratory bursting process to suppress the production of active oxygen species. The combination of pyruvate to neutralize active oxygen species and lactate to suppress the respiratory bursting process functions in a synergistic manner to reduce hydrogen peroxide production in the cell to levels lower than can be achieved by use of either type of component alone. The presence of mixtures of saturated and unsaturated fatty acids in the cytoprotective composition significantly enhances the ability of pyruvate and lactate to inhibit reactive oxygen production. Hence, the three components in the cytoprotective composition in this embodiment function together in a synergistic manner to prevent and reduce injury to mammalian cells, and increase the proliferation and resuscitation rate of the cells.

In a third embodiment, the therapeutic cytoprotective composition for treating mammalian cells, preferably epidermal keratinocytes, comprises (a) an antioxidant, and (b) a mixture of saturated and unsaturated fatty acids. The presence of mixtures of saturated and unsaturated fatty acids in the cytoprotective composition in this embodiment significantly enhances the ability of the antioxidant to inhibit reactive oxygen production. The combination of an antioxidant to neutralize active oxygen species and fatty acids to rebuild cellular membranes and reduce the need of the cell for oxygen functions in a synergistic manner to reduce hydrogen peroxide production in the cell to levels lower than can be achieved by either type of component alone. Hence, the components in the cytoprotective composition in this embodiment function together in a synergistic manner to prevent and reduce injury to mammalian cells, and increase the proliferation and resuscitation rate of the cells.

In a fourth embodiment, the therapeutic cytoprotective composition for treating mammalian cells, preferably monocytes, comprises (a) lactate, (b) an antioxidant, and (c) a mixture of saturated and unsaturated fatty acids. In this embodiment, lactate is employed because the respiratory bursting process is more pronounced in monocytes than in epidermal keratinocytes. The combination of lactate to suppress the respiratory bursting process and an antioxidant to neutralize active oxygen species functions in a synergistic manner to reduce hydrogen peroxide production in the cell to levels lower than can be achieved by either component alone. The presence of mixtures of saturated and unsaturated fatty acids in the cytoprotective composition in this embodiment significantly enhances the ability of lactate and the antioxidant to inhibit reactive oxygen production. Hence, the three components in the cytoprotective composition in this embodiment function together in a synergistic manner to prevent and reduce

injury to mammalian cells, and increase the proliferation and resuscitation rate of the cells.

In a fifth embodiment, the therapeutic cytoprotective composition for treating mammalian cells, preferably epidermal keratinocytes, comprises (a) pyruvate, and (b) an antioxidant. When the therapeutic cytoprotective composition in this embodiment is administered to cells before the cytotoxic agent is administered, the combination of pyruvate inside the cell and the antioxidant in the cellular membrane functions in a synergistic manner to reduce hydrogen peroxide production in the cell and thereby prevent injury to the cell. When injury to the cell is prevented and the cell does not require resuscitation, the mixture of saturated and unsaturated fatty acids need not be employed in the cytoprotective composition. Hence, the two components in the cytoprotective composition in this embodiment function together in a synergistic manner to prevent and reduce injury to mammalian cells.

Accordingly, the combination of ingredients set out in the above embodiments functions together in an enhanced manner to prevent and reduce injury to mammalian cells and to increase the proliferation and resuscitation rate of mammalian cells. The therapeutic effect of the combination of the components in each of the above embodiments is markedly greater than that expected by the mere addition of the individual therapeutic components. Hence, applicant's therapeutic cytoprotective compositions have the ability to decrease intracellular levels of hydrogen peroxide production, increase cellular resistance to cytotoxic agents, increase rates of cellular proliferation, and increase cellular viability.

The cells which may be treated with the cytoprotective compositions in the present invention are mammalian cells. Although applicant will describe the present cytoprotective compositions as useful for

treating mammalian epidermal keratinocytes and mammalian monocytes, applicant contemplates that all mammalian cells which may be protected or resuscitated by applicant's cytoprotective compositions may be used in the present invention. Keratinocytes are representative of normal mammalian cells and are the fastest proliferating cells in the body. The correlation between the reaction of keratinocytes to injury and therapy and that of mammalian cells in general is very high. Monocytes are representative of specialized mammalian cells such as the white blood cells in the immune system and the organ cells in liver, kidney, heart, and brain. The mammalian cells may be treated *in vivo* and *in vitro*.

Epidermal keratinocytes are the specialized epithelial cells of the epidermis which synthesize keratin, a scleroprotein which is the principal constituent of epidermis, hair, nails, horny tissue, and the organic matrix of the enamel of teeth. Mammalian epidermal keratinocytes constitute about 95% of the epidermal cells and together with melanocytes form the binary system of the epidermis. In its various successive stages, epidermal keratinocytes are also known as basal cells, prickle cells, and granular cells.

Monocytes are mononuclear phagocytic leukocytes which undergo respiratory bursting and are involved in reactive oxygen mediated damage within the epidermis. Leukocytes are white blood cells or corpuscles which may be classified into two main groups: granular leukocytes (granulocytes) which are leukocytes with abundant granules in the cytoplasm and nongranular leukocytes (nongranulocytes) which are leukocytes without specific granules in the cytoplasm and which include the lymphocytes and monocytes. Phagocyte cells are cells which ingest microorganisms or other cells and foreign particles. Monocytes are also known as large mononuclear leukocytes, and hyaline or transitional leukocytes.

Pyruvic acid (2-oxopropanoic acid, *alpha*-ketopropionic acid, $CH_3COCOOH$) or pyruvate (at physiological pH) is a fundamental intermediate in protein and carbohydrate metabolism and in the citric acid cycle. The citric acid cycle (tricarboxylic acid cycle, Kreb's cycle) is the major reaction sequence which executes the reduction of oxygen to generate adenosine triphosphate (ATP) by oxidizing organic compounds in respiring tissues to provide electrons to the transport system. Acetyl coenzyme A ("active acetyl") is oxidized in this process and is thereafter utilized in a variety of biological processes and is a precursor in the biosynthesis of many fatty acids and sterols. The two major sources of acetyl coenzyme A are derived from the metabolism of glucose and fatty acids. Glycolysis consists of a series of transformations wherein each glucose molecule is transformed in the cellular cytoplasm into two molecules of pyruvic acid. Pyruvic acid may then enter the mitochondria where it is oxidized by coenzyme A in the presence of enzymes and cofactors to acetyl coenzyme A. Acetyl coenzyme A can then enter the citric acid cycle.

In muscle, pyruvic acid (derived from glycogen) is reduced to lactic acid during exertion. Lactic acid is reoxidized and partially retransformed to glycogen during rest. Pyruvate can also act as an antioxidant to neutralize oxygen radicals in the cell and can be used in the multifunction oxidase system to reverse cytotoxicity.

The pyruvate in the present invention may be selected from the group consisting of pyruvic acid, pharmaceutically acceptable salts of pyruvic acid, and mixtures thereof. In general, the pharmaceutically acceptable salts of pyruvic acid may be alkali salts and alkaline earth salts. Preferably, the pyruvate is selected from the group consisting of pyruvic acid, sodium pyruvate, potassium pyruvate, magnesium pyruvate,

calcium pyruvate, zinc pyruvate, manganese pyruvate, and mixtures thereof. More preferably, the pyruvate is selected from the group of salts consisting of sodium pyruvate, potassium pyruvate, magnesium pyruvate, calcium pyruvate, zinc pyruvate, manganese pyruvate, and mixtures thereof. Most preferably, the pyruvate is sodium pyruvate.

The amount of pyruvate present in the cytoprotective compositions of the present invention is a therapeutically effective amount. A therapeutically effective amount of pyruvate is that amount of pyruvate necessary to increase the proliferation and resuscitation rate of mammalian cells. The exact amount of pyruvate is a matter of preference subject to such factors as the type of condition being treated as well as the other ingredients in the composition. When the cytoprotective composition contains two components, pyruvate is preferably present in the cytoprotective composition in an amount from about 10% to about 75%, preferably from about 20% to about 60%, and more preferably from about 25% to about 55%, by weight of the cytoprotective composition. When the cytoprotective composition contains three components, pyruvate is preferably present in the cytoprotective composition in an amount from about 10% to about 50%, preferably from about 20% to about 45%, and more preferably from about 25% to about 40%, by weight of the cytoprotective composition.

L-Lactic acid ((S)-2-hydroxypropanoic acid, (+) *alpha*-hydroxypropionic acid, $CH_3CHOHCOOH$) or lactate occurs in small quantities in the blood and muscle fluid of mammals. Lactic acid concentration increases in muscle and blood after vigorous activity. Lactate is a component in the cellular feedback mechanism and inhibits the natural respiratory bursting process of cells thereby suppressing the production of oxygen radicals.

The lactate in the present invention may be selected from the group consisting of lactic acid, pharmaceutically acceptable salts of lactic acid, and mixtures thereof. In general, the pharmaceutically acceptable salts of lactic acid may be alkali salts and alkaline earth salts. Preferably, the lactate is selected from the group consisting of lactic acid, sodium lactate, potassium lactate, magnesium lactate, calcium lactate, zinc lactate, manganese lactate, and mixtures thereof. More preferably, the lactate is selected from the group consisting of lactic acid, sodium lactate, potassium lactate, magnesium lactate, calcium lactate, zinc lactate, manganese lactate, and mixtures thereof. Most preferably, the lactate is lactic acid.

The amount of lactate present in the cytoprotective compositions of the present invention is a therapeutically effective amount. A therapeutically effective amount of lactate is that amount of lactate necessary to increase the proliferation and resuscitation rate of mammalian cells. For a composition, a therapeutically effective amount of lactate is that amount necessary to suppress the respiratory bursting process of white blood cells to protect and resuscitate the mammalian cells. In general, a therapeutically effective amount of lactate in a composition is from about 5 to about 10 times the amount of lactate normally found in serum. The exact amount of lactate is a matter of preference subject to such factors as the type of condition being treated as well as the other ingredients in the composition. In a preferred embodiment, lactate is present in the cytoprotective composition in an amount from about 10% to about 50%, preferably from about 20% to about 45%, and more preferably from about 25% to about 40%, by weight of the cytoprotective composition.

Antioxidants are substances which inhibit oxidation or suppress reactions promoted by oxygen or peroxides. Antioxidants, especially lipid-soluble

antioxidants, can be absorbed into the cellular membrane to neutralize oxygen radicals and thereby protect the membrane. The antioxidants useful in the present invention may be selected from the group consisting of Vitamin A (retinol), Vitamin A_2 (3, 4-didehydroretinol), all forms of carotene such as *alpha*-carotene, *beta*-carotene (*beta*, *beta*-carotene), *gamma*-carotene, *delta*-carotene, Vitamin C (ascorbic acid, L-ascorbic acid), all forms of tocopherol such as Vitamin E (*alpha*-tocopherol, 3,4-dihydro-2,5,7,8-tetramethyl-2-(4,8,12-trimethyltridecyl)-2H-1-benzopyran-6-ol), *beta*-tocopherol, *gamma*-tocopherol, and *delta*-tocopherol, and mixtures thereof. Preferably, the antioxidant is selected from the group of lipid-soluble antioxidants consisting of Vitamin A, *beta*-carotene, Vitamin E, and mixtures thereof. More preferably, the antioxidant is Vitamin E.

The amount of antioxidant present in the cytoprotective compositions of the present invention is a therapeutically effective amount. A therapeutically effective amount of antioxidant is that amount of antioxidant necessary to increase the proliferation and resuscitation rate of mammalian cells. The exact amount of antioxidant is a matter of preference subject to such factors as the type of condition being treated as well as the other ingredients in the composition. When the cytoprotective composition contains two components, the antioxidant is preferably present in the cytoprotective composition in an amount from about 10% to about 75%, preferably from about 20% to about 60%, and more preferably from about 25% to about 55%, by weight of the cytoprotective composition. When the cytoprotective composition contains three components, the antioxidant is preferably is present in the cytoprotective composition in an amount from about 10% to about 50%, preferably from about 20% to about 45%, and more preferably from about 25% to about 40%, by weight of the cytoprotective composition.

The mixture of saturated and unsaturated fatty acids in the present invention are those fatty acids required for the repair of mammalian cellular membranes and the production of new cells. Hence, the fatty acids are readily incorporated into the cell and are immediately available for the repair of injured cells and the proliferation of new cells. By providing the cell with the unsaturated fatty acids needed for repair, the need of the cell for unsaturated fatty acids is reduced and the need for high oxygen levels is also reduced. Accordingly, the presence of the mixtures of saturated and unsaturated fatty acids in the cytoprotective compositions significantly enhances the ability of pyruvate, lactate, and the antioxidant to inhibit reactive oxygen production.

Fatty acids are carboxylic acid compounds found in animal and vegetable fat and oil. Fatty acids are classified as lipids and are composed of chains of alkyl groups containing from 4 to 22 carbon atoms and 0-3 double bonds and characterized by a terminal carboxyl group, -COOH. Fatty acids may be saturated or unsaturated and may be solid, semisolid, or liquid. The most common saturated fatty acids are butyric acid (C_4), lauric acid (C_{12}), palmitic acid (C_{16}), and stearic acid (C_{18}). Unsaturated fatty acids are usually derived from vegetables and consist of alkyl chains containing from 16 to 22 carbon atoms and 0-3 double bonds with the characteristic terminal carboxyl group. The most common unsaturated fatty acids are oleic acid, linoleic acid, and linolenic acid (all C_{18} acids).

In general, the mixture of saturated and unsaturated fatty acids required for the repair of mammalian cellular membranes in the present invention may be derived from animal fats and waxes. Cells produce the chemical components and the energy required for cellular viability and store excess energy in the form of fat. Fat is adipose tissue stored between organs of the body

to furnish a reserve supply of energy. The preferred animal fats and waxes have a fatty acid composition similar to that of human fat and the fat contained in human breast milk. The preferred animal fats and waxes may be selected from the group consisting of human fat, chicken fat, cow fat (defined herein as a bovine domestic animal regardless of sex or age), sheep fat, horse fat, pig fat, and whale fat. The more preferred animal fats and waxes may be selected from the group consisting of human fat and chicken fat. The most preferred animal fat is human fat. Mixtures of other fats and waxes, such as vegetable waxes, marine oils (especially shark liver oil), and synthetic waxes and oils, which have a fatty acid composition similar to that of animal fats and waxes, and preferably to that of human fats and waxes, may also be employed. The mixture of saturated and unsaturated fatty acids may also be derived from animal and vegetable fats and waxes, and mixtures thereof.

In a preferred embodiment, the mixture of saturated and unsaturated fatty acids has a composition similar to that of human fat and comprises the following fatty acids: butyric acid, caproic acid, caprylic acid, capric acid, lauric acid, myristic acid, myristoleic acid, palmitic acid, palmitoleic acid, stearic, oleic acid, linoleic acid, linolenic acid, arachidic acid, and gaddoleic acid. Preferably, butyric acid, caproic acid, caprylic acid, capric acid, lauric acid, myristic acid, myristoleic acid, palmitic acid, palmitoleic acid, stearic, oleic acid, linoleic acid, linolenic acid, arachidic acid, and gaddoleic acid are present in the mixture in about the following percentages by weight, respectively (carbon chain number and number of unsaturations are shown parenthetically, respectively): 0.2%-0.4% (C_4), 0.1% (C_6), 0.3%-0.8% (C_8), 2.2%-3.5% (C_{10}), 0.9%-5.5% (C_{12}), 2.8%-8.5% (C_{14}), 0.1%-0.6% ($C_{14:1}$), 23.2%-24.6% (C_{16}), 1.8%-3.0% ($C_{16:1}$), 6.9%-9.9% (C_{18}), 36.0%-36.5% ($C_{18:1}$), 20%-20.6% ($C_{18:2}$), 7.5-7.8% ($C_{18:3}$), 1.1%-4.9% (C_{20}), and 3.3%-6.4% ($C_{20:1}$).

In another preferred embodiment, the mixture of saturated and unsaturated fatty acids is typically chicken fat comprising the following fatty acids: lauric acid, myristic acid, myristoleic acid, pentadecanoic acid, palmitic acid, palmitoleic acid, margaric acid, margaroleic acid, stearic, oleic acid, linoleic acid, linolenic acid, arachidic acid, and gaddoleic acid. Preferably, lauric acid, myristic acid, myristoleic acid, pentadecanoic acid, palmitic acid, palmitoleic acid, margaric acid, margaroleic acid, stearic, oleic acid, linoleic acid, linolenic acid, arachidic acid, and gaddoleic acid are present in the mixture in about the following percentages by weight, respectively: 0.1% (C_{12}), 0.8% (C_{14}), 0.2% ($C_{14:1}$), 0.1% (C_{15}), 25.3% (C_{16}), 7.2% ($C_{16:1}$), 0.1% (C_{17}), 0.1% ($C_{17:1}$), 6.5% (C_{18}), 37.7% ($C_{18:1}$), 20.6% ($C_{18:2}$), 0.8% ($C_{18:3}$), 0.2% (C_{20}), and 0.3% ($C_{20:1}$), all percentages +/- 10%.

The above fatty acids and percentages thereof present in the fatty acid mixture are given as an example. The exact type of fatty acid present in the fatty acid mixture and the exact amount of fatty acid employed in the fatty acid mixture may be varied in order to obtain the result desired in the final product and such variations are now within the capabilities of those skilled in the art without the need for undue experimentation.

The amount of fatty acids present in the cytoprotective compositions of the present invention is a therapeutically effective amount. A therapeutically effective amount of fatty acids is that amount of fatty acids necessary to increase the repair of cellular membranes and resuscitation rate of mammalian cells. The exact amount of fatty acids employed is subject to such factors as the type and distribution of fatty acids employed in the mixture, the type of condition being treated, and the other ingredients in the composition.

When the cytoprotective composition contains two components, the fatty acids are preferably present in the cytoprotective composition in an amount from about 10% to about 75%, preferably from about 20% to about 60%, and more preferably from about 25% to about 55%, by weight of the cytoprotective composition. When the cytoprotective composition contains three components, the fatty acids are preferably present in the cytoprotective composition in an amount from about 10% to about 50%, preferably from about 20% to about 45%, and more preferably from about 25% to about 40%, by weight of the cytoprotective composition.

In accord with the present invention, the therapeutic cytoprotective compositions for preventing and reducing injury to mammalian cells from a medicament having cytotoxic properties may be selected from the group consisting of:

(1) (a) pyruvate selected from the group consisting of pyruvic acid, pharmaceutically acceptable salts of pyruvic acid, and mixtures thereof;

(b) an antioxidant; and

(c) a mixture of saturated and unsaturated fatty acids wherein the fatty acids are those fatty acids required for the repair of cellular membranes and resuscitation of mammalian cells;

(2) (a) pyruvate selected from the group consisting of pyruvic acid, pharmaceutically acceptable salts of pyruvic acid, and mixtures thereof;

(b) lactate selected from the group consisting of lactic acid, pharmaceutically acceptable salts of lactic acid, and mixtures thereof; and

(c) a mixture of saturated and unsaturated fatty acids wherein the fatty acids are those fatty acids required for the repair of cellular membranes and resuscitation of mammalian cells;

(3) (a) an antioxidant; and

(b) a mixture of saturated and unsaturated fatty acids wherein the fatty acids are those fatty acids

required for the repair of cellular membranes and resuscitation of mammalian cells;

(4) (a) lactate selected from the group consisting of lactic acid, pharmaceutically acceptable salts of lactic acid, and mixtures thereof;

(b) an antioxidant; and

(c) a mixture of saturated and unsaturated fatty acids wherein the fatty acids are those fatty acids required for the repair of cellular membranes and resuscitation of mammalian cells; and

(5) (a) pyruvate selected from the group consisting of pyruvic acid, pharmaceutically acceptable salts of pyruvic acid, and mixtures thereof; and

(b) an antioxidant.

In a preferred embodiment, the cytoprotective compositions for treating mammalian cells, preferably epidermal keratinocytes, may be selected from the group consisting of:

(1) (a) pyruvate selected from the group consisting of pyruvic acid, pharmaceutically acceptable salts of pyruvic acid, and mixtures thereof;

(b) an antioxidant; and

(c) a mixture of saturated and unsaturated fatty acids wherein the fatty acids are those fatty acids required for the repair of cellular membranes and resuscitation of mammalian cells;

(2) (a) pyruvate selected from the group consisting of pyruvic acid, pharmaceutically acceptable salts of pyruvic acid, and mixtures thereof;

(b) lactate selected from the group consisting of lactic acid, pharmaceutically acceptable salts of lactic acid, and mixtures thereof; and

(c) a mixture of saturated and unsaturated fatty acids wherein the fatty acids are those fatty acids required for the repair of cellular membranes and resuscitation of mammalian cells; and

(3) (a) an antioxidant; and

(b) a mixture of saturated and unsaturated fatty acids wherein the fatty acids are those fatty acids required for the repair of cellular membranes and resuscitation of mammalian cells; and

(5) (a) pyruvate selected from the group consisting of pyruvic acid, pharmaceutically acceptable salts of pyruvic acid, and mixtures thereof; and

(b) an antioxidant.

In a more preferred embodiment, the cytoprotective compositions for treating mammalian cells, preferably epidermal keratinocytes, may be selected from the group consisting of:

(1) (a) pyruvate selected from the group consisting of pyruvic acid, pharmaceutically acceptable salts of pyruvic acid, and mixtures thereof;

(b) an antioxidant; and

(c) a mixture of saturated and unsaturated fatty acids wherein the fatty acids are those fatty acids required for the repair of cellular membranes and resuscitation of mammalian cells; and

(5) (a) pyruvate selected from the group consisting of pyruvic acid, pharmaceutically acceptable salts of pyruvic acid, and mixtures thereof; and

(b) an antioxidant.

In a most preferred embodiment, the cytoprotective compositions for treating mammalian cells, preferably epidermal keratinocytes, comprise:

(a) pyruvate selected from the group consisting of pyruvic acid, pharmaceutically acceptable salts of pyruvic acid, and mixtures thereof;

(b) an antioxidant; and

(c) a mixture of saturated and unsaturated fatty acids wherein the fatty acids are those fatty acids required for the repair of cellular membranes and resuscitation of mammalian cells.

The present invention extends to methods for making the therapeutic cytoprotective compositions. In general, a cytoprotective composition is made by forming an admixture of the components of the composition. In a first embodiment, a cytoprotective composition is made by forming an admixture of (a) a pyruvate, (b) an antioxidant, and (c) a mixture of saturated and unsaturated fatty acids. In a second embodiment, a cytoprotective composition is made by forming an admixture of (a) a pyruvate, (b) a lactate, and (c) a mixture of saturated and unsaturated fatty acids. In a third embodiment, a cytoprotective composition is made by forming an admixture of (a) an antioxidant, and (b) a mixture of saturated and unsaturated fatty acids. In a fourth embodiment, a cytoprotective composition is made by forming an admixture of (a) a lactate, (b) an antioxidant, and (c) a mixture of saturated and unsaturated fatty acids. In a fifth embodiment, a cytoprotective composition is made by forming an admixture of (a) a pyruvate, and (b) an antioxidant.

For some applications, the admixture may be formed in a solvent such as water. If necessary, the pH of the solvent is adjusted to a range from about 3.5 to about 8.0, and preferably from about 4.5 to about 7.5, and more preferably about 6.0 to about 7.4. The admixture is then sterile filtered. Other ingredients may also be incorporated into the cytoprotective composition as dictated by the nature of the desired composition as well known by those having ordinary skill in the art. The ultimate cytoprotective compositions are readily prepared using methods generally known in the pharmaceutical arts.

Once prepared, the inventive therapeutic cytoprotective compositions may be stored for future use or may be formulated in effective amounts with a cytotoxic agent to form cytoprotective pharmaceutical compositions. The combination of the cytoprotective

compositions of the present invention and the medicament cytotoxic to cells provides a cytoprotective pharmaceutical composition having the ability to prevent and reduce injury to mammalian cells from the cytotoxic medicament and increase the resuscitation rate of injured mammalian cells. The dose level of the cytotoxic medicament in the cytoprotective pharmaceutical composition may thereby be raised to higher than normal levels.

The cytotoxic agents which may be used in the cytoprotective pharmaceutical compositions of the present invention may be selected from a wide variety of medicaments. For example, medicaments taken on a long term regimen tend to cause liver, kidney, tissue, and other toxicity problems. In addition, certain cytotoxic medicaments, such as potent chemotherapeutic medicaments used to treat malignant tissues, are believed to stimulate release of significant amounts of reactive oxygen species by mammalian tissues which can cause oxidative injury. Combination of the cytoprotective compositions of the present invention with such cytotoxic medicaments may inhibit induction of reactive oxygen production while simultaneously decreasing side effects of such medicaments. By decreasing the side effects of such medicaments, the dosage levels of the medicaments may be increased thereby increasing the therapeutic effect of the medicaments. For example, the cytoprotective compositions may be used in topical cytoprotective pharmaceutical compositions in combination with cytotoxic medicaments such as epithelial cell cohesiveness reducers such as tretinoin (Retin A), dermatological abradants, and anti-inflammatories, to protect and enhance the resuscitation rate of the injured mammalian cells. The cytoprotective compositions may also be used in ingestible cytoprotective pharmaceutical compositions in combination with medicaments that cause cytotoxic side effects such as anti-tumor, anti-viral, and antibacterial medicaments including the lipid

regulating agents gemfibrozil and lovastatin, centrally acting anticholinesterases such as tacrine, chemotherapeutic medicaments such as the anthracycline antibiotic doxorubicin, gastric irritants such as acetylsalicylic acid and ibuprofen, to protect and enhance the resuscitation rate of the injured mammalian cells.

In one preferred embodiment, the medicament having cytotoxic properties in the cytoprotective pharmaceutical compositions is selected from the group consisting of doxorubicin, gemfibrozil, lovastatin, and tacrine. In a more preferred embodiment, the medicament having cytotoxic properties in the cytoprotective pharmaceutical compositions is selected from the group consisting of doxorubicin, gemfibrozil, and tacrine. In a most preferred embodiment, the medicament having cytotoxic properties is doxorubicin. Doxorubicin (Adriamycin) is a cytotoxic anthracycline antibiotic reported to produce regression in disseminated neoplastic conditions such as in various leukemias, tumors, neuroblastomas, sarcomas, and carcinomas. Gemfibrozil (Lopid) is a lipid regulating agent which lowers elevated serum lipids primarily by decreasing serum triglyceride with a variable reduction in total serum cholesterol. Lovastatin (Mevacor) is a cholesterol lowering agent which inhibits the enzymatic biosynthesis of cholesterol. Tacrine (Cognex, 1,2,3,4-tetrahydro-9-acridinamine) is a centrally active anticholinesterase useful as a cognition activator. Tacrine has undergone clinical trials for use in treating severe Alzheimer's disease (presenile dementia).

In another preferred embodiment, the medicament having cytotoxic properties in the cytoprotective pharmaceutical compositions is an anticancer agent. Nonlimiting examples of anticancer agents include chemically reactive drugs having nonspecific action, anti-metabolites, antibiotics, plant products, hormones,

and other miscellaneous chemotherapeutic agents. Chemically reactive drugs having nonspecific action include alkylating agents and N-alkyl-N-nitroso compounds. Examples of alkylating agents include nitrogen mustards, azridines (ethylenimines), sulfonic acid esters, and epoxides. Anti-metabolites are compounds that interfere with the formation or utilization of a normal cellular metabolite and include amino acid antagonists, vitamin and coenzyme antagonists, and antagonists of metabolites involved in nucleic acid synthesis such as glutamine antagonists, folic acid antagonists, pyrimidine antagonists, and purine antagonists. Antibiotics are compounds produced by microorganisms that have the ability to inhibit the growth of other organisms and include actinomycins and related antibiotics, glutarimide antibiotics, sarkomycin, fumagillin, streptonigrin, tenuazonic acid, actinogan, peptinogan, and anthracyclic antibiotics such as doxorubicin. Plant products include colchicine, podophyllotoxin, and vinca alkaloids. Hormones include those steroids used in breast and prostate cancer and corticosteroids used in leukemias and lymphomas. Other miscellaneous chemotherapeutic agents include urethan, hydroxyurea, and related compounds; thiosemicarbazones and related compounds; phthalanilide and related compounds; and triazenes and hydrazines. In a preferred embodiment, the anticancer agent is an antibiotic. In a more preferred embodiment, the anticancer agent is doxorubicin. In a most preferred embodiment, the anticancer agent is doxorubicin.

In a specific embodiment, the invention is directed at a cytoprotective pharmaceutical composition for preventing and reducing injury to mammalian cells from a medicament having cytotoxic properties which comprises:

(A) a cytoprotective composition selected from the group consisting of:

(1) (a) pyruvate selected from the group consisting of pyruvic acid, pharmaceutically acceptable salts of pyruvic acid, and mixtures thereof;

(b) an antioxidant; and

(c) a mixture of saturated and unsaturated fatty acids wherein the fatty acids are those fatty acids required for the resuscitation of injured mammalian cells; and

(2) (a) pyruvate selected from the group consisting of pyruvic acid, pharmaceutically acceptable salts of pyruvic acid, and mixtures thereof; and

(b) an antioxidant; and

(B) a medicament having cytotoxic properties.

In another form of this embodiment, the cytoprotective compositions of the present invention, may be combined in an immediate release form with an anticancer medicament having cytotoxic properties in a timed release form to provide a timed release cytoprotective pharmaceutical composition. In this embodiment, the timed release composition releases the cytoprotective composition substantially immediately and releases the cytotoxic chemotherapeutic medicament after a suitable period of time, for example from one to 24 hours after releasing the cytoprotective composition, to selectively protect non-cancerous cells in the presence of cancerous cells against the cytotoxic chemotherapeutic medicament. Cancer cells, unlike normal cells or benign tumor cells, exhibit the properties of invasion and metastasis and are highly anaplastic. Because cancerous cells have a rapid metabolism, cancerous cells will rapidly consume the protective cytoprotective composition and will not be protected by the cytoprotective compositions when the chemotherapeutic medicament is subsequently released. Non-cancerous cells which do not have such a rapid metabolism will not rapidly consume the cytoprotective compositions and will be protected when the chemotherapeutic medicament is subsequently released.

In a specific embodiment, the invention is direct at a timed-release cytoprotective pharmaceutical composition for selectively protecting non-cancerous mammalian cells in the presence of cancerous mammalian cells from an anticancer medicament having cytotoxic properties which comprises:

(A) a cytoprotective composition in immediate release form selected from the group consisting of:

(1) (a) pyruvate selected from the group consisting of pyruvic acid, pharmaceutically acceptable salts of pyruvic acid, and mixtures thereof;

(b) an antioxidant; and

(c) a mixture of saturated and unsaturated fatty acids wherein the fatty acids are those fatty acids required for the resuscitation of injured mammalian cells; and

(2) (a) pyruvate selected from the group consisting of pyruvic acid, pharmaceutically acceptable salts of pyruvic acid, and mixtures thereof; and

(b) an antioxidant; and

(B) an anticancer medicament having cytotoxic properties in timed-release form;

wherein the cytoprotective composition is released substantially immediately and the anticancer medicament is released after a period of time sufficient such that the cancerous cells have substantially metabolized the cytoprotective composition and the non-cancerous cells have not substantially metabolized the cytoprotective composition.

A suitable or sufficient period of time is that period of time wherein the cancerous cells have substantially metabolized the cytoprotective composition and the non-cancerous cells have not substantially metabolized the cytoprotective composition. The period of time should not be so long that the non-cancerous cells substantially metabolize the cytoprotective composition and are unprotected. The exact time is

subject to such factors as the type and quantity of cytoprotective composition employed, the medicament having cytotoxic properties used, and the type of cancerous cells and non-cancerous cells being treated. Thus, the period of time may be varied in order to obtain the result desired and such variations are within the capabilities of those skilled in the art without the need for undue experimentation.

The present invention extends to methods for making the cytoprotective pharmaceutical composition. In general, a cytoprotective pharmaceutical composition is made by forming an admixture of the components of the composition. The cytoprotective compositions may be prepared using standard techniques and equipment known to those skilled in the art. The apparatus useful in accordance with the present invention comprises apparatus well known in the chemical and biochemical arts, and therefore the selection of the specific apparatus will be apparent to the artisan.

In one embodiment, a cytoprotective pharmaceutical composition is made by forming an admixture of the cytoprotective composition and the medicament having cytotoxic properties. In a second embodiment, a timed-release cytoprotective pharmaceutical composition is made by forming an admixture of the cytoprotective composition in immediate release form and the anticancer medicament having cytotoxic properties in timed-release form.

The present invention extends to methods for using the therapeutic cytoprotective compositions. In one embodiment, the cytoprotective compositions of the present invention may be administered to cells concurrently with a cytotoxic medicament. In another embodiment, the cytoprotective compositions of the present invention may be administered to cells prior to the administration of a cytotoxic anticancer medicament

to selectively protect non-cancerous cells in the presence of cancerous cells against the anticancer agent.

In a specific embodiment, the invention is directed at a method for protecting mammalian cells from a medicament having cytotoxic properties which comprises the steps of:

(A) providing a cytoprotective composition selected from the group consisting of:

(1) (a) pyruvate selected from the group consisting of pyruvic acid, pharmaceutically acceptable salts of pyruvic acid, and mixtures thereof;

(b) an antioxidant; and

(c) a mixture of saturated and unsaturated fatty acids wherein the fatty acids are those fatty acids required for the resuscitation of injured mammalian cells; and

(2) (a) pyruvate selected from the group consisting of pyruvic acid, pharmaceutically acceptable salts of pyruvic acid, and mixtures thereof; and

(b) an antioxidant; and

(B) providing an anticancer medicament having cytotoxic properties; and

(C) administering the cytoprotective composition from step (A) and the medicament from step (B) concurrently to mammalian cells to protect the mammalian cells from the medicament having cytotoxic properties.

In another specific embodiment, the invention is direct at a method for selectively protecting non-cancerous mammalian cells in the presence of cancerous mammalian cells from an anticancer medicament having cytotoxic properties which comprises the steps of:

(A) providing a cytoprotective composition in an immediate release form selected from the group consisting of:

(1) (a) pyruvate selected from the group consisting of pyruvic acid, pharmaceutically acceptable salts of pyruvic acid, and mixtures thereof;

(b) an antioxidant; and

(c) a mixture of saturated and unsaturated fatty acids wherein the fatty acids are those fatty acids required for the resuscitation of injured mammalian cells; and

(2) (a) pyruvate selected from the group consisting of pyruvic acid, pharmaceutically acceptable salts of pyruvic acid, and mixtures thereof; and

(b) an antioxidant; and

(B) providing an anticancer medicament having cytotoxic properties in timed-release form; and

(C) administering the cytoprotective composition from step (A) and the medicament from step (B) concurrently to mammalian cells to selectively protect non-cancerous mammalian cells in the presence of cancerous mammalian cells from the anticancer medicament having cytotoxic properties;

wherein the cytoprotective composition is released substantially immediately and the anticancer medicament is released after a period of time sufficient such that the cancerous cells have substantially metabolized the cytoprotective composition and the non-cancerous cells have not substantially metabolized the cytoprotective composition.

In yet another specific embodiment, the invention is direct at a method for selectively protecting non-cancerous mammalian cells in the presence of cancerous mammalian cells from an anticancer medicament having cytotoxic properties which comprises the steps of:

(A) administering to mammalian cells a cytoprotective composition to prevent and reduce injury to the mammalian cells selected from the group consisting of:

(1) (a) pyruvate selected from the group consisting of pyruvic acid, pharmaceutically acceptable salts of pyruvic acid, and mixtures thereof;

(b) an antioxidant; and

(c) a mixture of saturated and unsaturated fatty acids wherein the fatty acids are those fatty acids required for the resuscitation of injured mammalian cells; and

(2) (a) pyruvate selected from the group consisting of pyruvic acid, pharmaceutically acceptable salts of pyruvic acid, and mixtures thereof; and

(b) an antioxidant; and

(B) waiting a period of time sufficient such that the cancerous cells have substantially metabolized the cytoprotective composition and the non-cancerous cells have not substantially metabolized the cytoprotective composition; and

(C) administering the cytotoxic anticancer medicament to the mammalian cells to treat the cancerous cells which are unprotected by the cytoprotective composition and the non-cancerous cells which are protected by the cytoprotective composition to thereby increase the therapeutic effect of the anticancer medicament.

Methods for administering the cytoprotective compositions of the present invention to mammalian cells will vary depending upon the particular condition being treated and the cytotoxic agent employed. In general, the cytoprotective compositions will be administered in the same manner as the cytotoxic agent. Of course, the type of carrier will vary depending upon the mode of administration desired for the pharmaceutical composition as is conventional in the art.

The cytoprotective compositions of the present invention may be administered parenterally, in the form of sterile solutions or suspensions, such as intravenously, intramuscularly, or subcutaneously. The cytoprotective compositions may also be administered topically. Non-oral topical compositions employ non-oral topical vehicles, such as oils, petrolatum bases, emulsions, lotions, creams, gel formulations, foams,

ointments, sprays, salves, and films, which are intended to be applied to the skin or body cavity and are not intended to be taken by mouth. Oral topical compositions employ oral vehicles, such as mouthwashes, rinses, oral sprays, suspensions, bioadhesives, and dental gels, which are intended to be taken by mouth but are not intended to be ingested. The cytoprotective compositions may also be administered orally, in the form of pills, tablets, capsules, troches, and the like, as well as sublingually, rectally, or transcutaneously with a suitable pharmaceutically acceptable carrier for that particular mode of administration as is conventional in the art.

It is especially advantageous to formulate the pharmaceutical compositions in dosage unit forms for ease of administration and uniformity of dosage. The term dosage unit forms as used herein refers to physically discrete units suitable for use as a unitary dosage, each unit containing a predetermined quantity of active ingredient calculated to produce the desired therapeutic effect in association with the pharmaceutical carrier.

For parental therapeutic administration, the cytoprotective compositions of the present invention may be incorporated into a sterile solution or suspension. These preparations should contain at least about 0.1% of the inventive composition, by weight, but this amount may be varied to between about 0.1% and about 50% of the inventive composition, by weight of the parental composition. The exact amount of the inventive composition present in such compositions is such that a suitable dosage level will be obtained. Preferred compositions and preparations according to the present invention are prepared so that a paranteral dosage unit contains from between about 0.5 milligrams to about 100 milligrams of the inventive composition.

Suitable carriers include propylene glycol-alcohol-water, isotonic water, sterile water for

injection (USP), emulphor^{TM}-alcohol-water, cremophor-EL^{TM} or other suitable carriers known to those skilled in the art. The sterile solutions or suspensions may also include the following adjuvants: a sterile diluent, such as water for injection, saline solution, fixed oils, polyethylene glycol, glycerine, propylene glycol, or other synthetic solvent; antibacterial agents, such as benzyl alcohol or methyl paraben; antioxidants, such as ascorbic acid or sodium metabisulfite; chelating agents, such as ethylenediaminetetraacetic acid (EDTA); buffers, such as acetates, citrates or phosphates; and agents for the adjustment of tonicity, such as sodium chloride or dextrose. The parental preparations may be enclosed in ampules, disposable syringes, or multiple dose vials made of glass or plastic.

In another form of the invention, the therapeutic cytoprotective composition is incorporated into a non-oral topical vehicle which may be in the form of oils, petrolatum bases, emulsions, lotions, creams, gels formulations, foams, ointments, sprays, salves, and films, and the like. Non-oral topical vehicles include water and pharmaceutically acceptable water-miscible organic solvents such as ethyl alcohol, isopropyl alcohol, propylene glycol, glycerin, and the like, and mixtures of these solvents. Typical non-toxic non-oral topical vehicles known in the pharmaceutical arts may be used. The non-oral topical cytoprotective compositions may also contain conventional additives employed in those products. Conventional additives include humectants, emollients, lubricants, stabilizers, dyes, and perfumes, providing the additives do not interfere with the therapeutic properties of the cytoprotective composition.

In another form of the invention, the cytoprotective composition is incorporated into an oral topical vehicle which may be in the form of a mouthwash, rinse, oral spray, suspension, dental gel, bioadhesive, and the like. Typical non-toxic oral vehicles known in

the pharmaceutical arts may be used in the present invention. The preferred oral vehicles are water, ethanol, and water-ethanol mixtures. The water-ethanol mixtures are generally employed in a weight ratio from about 1:1 to about 20:1, preferably from about 3:1 to about 20:1, and most preferably from about 3:1 to about 10:1, respectively. The pH value of the oral vehicle is generally from about 4 to about 7, and preferably from about 5 to about 6.5. An oral topical vehicle having a pH value below about 4 is generally irritating to the oral cavity and an oral vehicle having a pH value greater than about 7 generally results in an unpleasant mouth feel. The oral topical cytoprotective compositions may also contain conventional additives normally employed in those products. Conventional additives include a fluorine providing compound, a sweetening agent, a flavoring agent, a coloring agent, a humectant, a buffer, and an emulsifier, providing the additives do not interfere with the therapeutic properties of the cytoprotective composition.

In accordance with this invention, therapeutically effective amounts of the cytoprotective compositions of the present invention may be admixed with a topical vehicle to form a topical cytoprotective composition. These amounts are readily determined by those skilled in the art without the need for undue experimentation. In a preferred embodiment, the topical cytoprotective compositions will comprise the cytoprotective composition in an amount from about 0.1% to about 10% and a topical vehicle in a quantity sufficient to bring the total amount of composition to 100%, by weight of the topical cytoprotective composition. In a more preferred embodiment, the topical cytoprotective compositions will comprise the cytoprotective composition in an amount from about 0.1% to about 10%, and in a most preferred embodiment, the topical cytoprotective compositions will comprise the cytoprotective composition in an amount from about 0.1%

to about 8%, and a topical vehicle in a quantity sufficient to bring the total amount of composition to 100%, by weight of the topical cytoprotective composition.

The present invention extends to methods for preparing the topical cytoprotective compositions. In such a method, the topical cytoprotective composition is prepared by admixing a therapeutically effective amount of the cytoprotective composition of the present invention, the cytotoxic agent, and a topical vehicle. The final compositions are readily prepared using standard methods and apparatus generally known by those skilled in the pharmaceutical arts. The apparatus useful in accordance with the present invention comprises mixing apparatus well known in the pharmaceutical arts, and therefore the selection of the specific apparatus will be apparent to the artisan.

In a specific embodiment, the invention is directed at a cytoprotective pharmaceutical composition which comprises a pharmaceutically acceptable carrier and a therapeutically effective amount of a cytoprotective composition for preventing and reducing injury to mammalian cells from a medicament having cytotoxic properties, wherein the cytoprotective composition is selected from the group consisting of:

(1) (a) pyruvate selected from the group consisting of pyruvic acid, pharmaceutically acceptable salts of pyruvic acid, and mixtures thereof;

(b) an antioxidant; and

(c) a mixture of saturated and unsaturated fatty acids wherein the fatty acids are those fatty acids required for the resuscitation of injured mammalian cells; and

(2) (a) pyruvate selected from the group consisting of pyruvic acid, pharmaceutically acceptable salts of pyruvic acid, and mixtures thereof; and

(b) an antioxidant.

In another specific embodiment, the invention is directed at a method for preparing a cytoprotective pharmaceutical composition for protecting mammalian cells from a medicament having cytotoxic properties which comprises the steps of:

(A) providing a therapeutically effective amount of a cytoprotective composition which comprises:

(1) (a) pyruvate selected from the group consisting of pyruvic acid, pharmaceutically acceptable salts of pyruvic acid, and mixtures thereof;

(b) an antioxidant; and

(c) a mixture of saturated and unsaturated fatty acids wherein the fatty acids are those fatty acids required for the resuscitation of injured mammalian cells; and

(2) (a) pyruvate selected from the group consisting of pyruvic acid, pharmaceutically acceptable salts of pyruvic acid, and mixtures thereof; and

(b) an antioxidant; and

(B) providing a pharmaceutically acceptable carrier; and

(C) admixing the cytoprotective composition from step (A) and the pharmaceutically acceptable carrier from step (B) to form a pharmaceutical composition.

Throughout this application, various publications have been referenced. The disclosures in these publications are incorporated herein by reference in order to more fully describe the state of the art.

The present invention is further illustrated by the following examples which are not intended to limit the effective scope of the claims. All parts and percentages in the examples and throughout the specification and claims are by weight of the final composition unless otherwise specified.

EXAMPLES

These examples demonstrate the cytoprotective abilities of the therapeutic cytoprotective compositions of the present invention.

Methods

Isolation of Peripheral Blood Monocytes

Peripheral blood was obtained from a normal healthy volunteer by venipuncture using an EDTA-containing Vacutainer (Becton Dickinson Mountain View, Ca.). A total of 10 ml of peripheral blood was mixed in a ratio of 1:1 with Dulbecco's Minimal Essential Medium (DMEM, Grand Island Biologicals, GIBCO, Grand Island, N.Y.). The mixture was divided into 2 ml portions and each portion was layered onto 6 ml of Ficoll-Hypaque gradient mixture (Pharmacy, Inc., Piscataway, N.J.) and centrifuged in a Beckman T-J6 refrigerated centrifuge for 30 minutes at 1500 rpm and 4° C. After the cells were washed twice with phosphate buffered saline, the cells were resuspended in Hank's Balanced Salt Solution without Ca^{++}/Mg^{++} (GIBCO).

Culture of U937 and Peripheral Blood Monocytes

Peripheral blood monocytes and U937 monocytic leukemia tumor cells were placed in sterile culture flasks and maintained in culture using Dulbecco's Minimal Essential Medium, with 10% fetal calf serum, supplemented with 2mM glutamine and Pen/Strep. The cytotoxicity of the cytotoxic agent on the cells was analyzed by propidium iodide exclusion techniques and flow cytometric quantitation. Viability of the cells was quantified as the number of cells that excluded the vital dye trypan blue.

Preparation of Chemicals

Sodium pyruvate was dissolved in distilled water and the solution was adjusted to pH 7.4 with 1N sodium hydroxide solution. Solutions were sterile filtered. Stock solutions were prepared so that the vehicle would not be more than 1% of the total volume of the culture media.

A mixture of fatty acids derived from chicken fat was prepared by mixing 0.1% chicken fat with mineral oil to form an emulsified solution. Tween 80 was added to separate cultures of cells at similar concentrations and to examine possible vehicle effects.

Alpha-tocopherol phosphate (Sigma Chemical Company, St. Louis, MO) was added directly to the culture medium.

^{3}H-Thymidine Radiosotopic Incorporation
Measurement of Cytotoxity

Cells were plated into 96 well dishes at a concentration of 10^{6} cells/well. Tritiated thymidine (1 uCi/well) was added and the cells were incubated for 4 hours at which time the cells were harvested using a Cambridge cell harvestor. The samples were then placed in scintillation vials containing scintillation fluid and counted. These studies yielded a measure of the ability of the cells to proliferate, which is a measure of viability.

The results from the tritiated thymidine incorporation assay, a measure of DNA synthesis and cellular proliferation, correlated directly with the results from the dye exclusion viability assay. Because the tritiated thymidine incorporation assay is a more quantitative assay, the tritiated thymidine incorporation assay was used for the remainder of the studies.

A dose response curve for Doxorubicin (Adriamycin) alone was constructed. Doxorubicin is an anthracycline antibiotic used as a first line agent in a number of neoplastic conditions and is a well characterized cytotoxic agent. Doses and times examined ranged from 0.1, 0.5, 1, 5, 10, 25, and 50 ug/ml of Doxorubicin for 20-60 minutes and 24 hours. The range of optimal concentrations for cytotoxity of Doxorubicin was established for u937 monocytic tumor cells to be 0.5, 1 and 5 ug at 24 hours and 10 ug at 1-2 hours, see Figures 1 and 2.

The cytoprotective agents (sodium pyruvate, Vitamin E, and fatty acids) alone, and in combination, were examined for their ability to decrease the cytotoxity of Doxorubicin to U937 monocytic leukemia cells and normal peripheral blood monocytes. Optimal concentrations of the single ingredients of sodium pyruvate, Vitamin E, and fatty acids were examined. The optimal concentrations of the agents that were able to protect cells against Doxorubicin induced cytotoxity were as follows: 10-50 U Vitamin E, 0.5% fatty acids, and 5 mM of sodium pyruvate, see Figure 3.

Window of susceptibility studies were conducted to determine the optimal treatment time of the cells with the cytoprotective agents prior to treatment of the cells with the cytotoxic agent. The normal cells and U937 leukemic tumor cells were pretreated separately in "wash out" studies with the single agents alone, and in combination, at the optimal concentration described above for various time periods, washed with fresh medium to remove the agents, and treated with the cytotoxic agent. The co-culture of normal and U937 leukemic tumor cells was treated essentially in the same manner except that the cells were not treated separately, but co-cultured. The optimal pretreatment time of the cells with the cytoprotective agents was found to be 24 hours prior to

treatment of the cells with Doxorubicin. The cells were then placed in culture medium without the protective agents. The length of time that the cytoprotection lasted was 24 hours following Doxorubicin treatment. At this time, peripheral cell viability is a limiting factor because these cells are normal cells and do not remain in culture for extended periods of time.

Normal and U937 tumor cells were co-cultured and the cytotoxity of Doxorubicin on the cells was determined by viability assays which examined the differential ability of the cytoprotective compositions alone, and in combinations, to protect the normal cells from the cytotoxity of the chemotherapeutic agent.

The cells were isolated and examined for morphological evidence of cytotoxicity or prevention of cytotoxicity. These studies determined the cytoprotective effect of the single agents and the combination of agents on the normal and tumor cells. DNA synthesis studies using ^{3}H-thymidine (1 uCi/well) were carried out 4 hours prior to termination of the experiment to determine the effect of the formulations on the proliferation of the cells as a measure of the prevention of cytotoxicity and the extent of Doxorubicin-induced cytotoxicity. Propidium iodide exclusion analysis was carried out for direct quantitation of the cytotoxicity and the prevention of cytotoxicity. Each set of studies was performed in triplicate so that statistical analysis of the significant differences between the treatment groups could be conducted.

The effect of the cytoprotective agents on the co-culture of tumor and normal cells was very different from the effect of these agents on the individual cell types alone. An interaction between the normal cells and the tumor cells must cause the viability of the tumor cells to be significantly diminished. The cytoprotective combination of 5 mM sodium pyruvate, 0.5% fatty acids,

and 10 U Vitamin E provided significant protection to the normal peripheral monocytes and did not protect the tumor cells from the effects of the cytotoxic agent.

Wash-out studies were conducted to determine viability of the peripheral blood monocytes co-cultured with U937 monocytic leukemia cells after 24 hour pretreatment of the cells with the cytoprotective agents followed by administration of Doxorubicin. With no Doxorubicin treatment, the viability of the control normal peripheral cells was enhanced from 55% to 68% with the use of 5 mM sodium pyruvate and 0.5% fatty acids, see Figure 3. With no Doxorubicin treatment, the viability of the control U937 cells was enhanced from 43% to 62% with the use of the combination of the cytoprotective composition, 5 mM sodium pyruvate, 10 U Vitamin E, and 0.5% fatty acids, see Figure 3.

Pretreatment with a combination of 10 U Vitamin E and 5 mM sodium pyruvate prevented cytotoxity to normal peripheral blood monocytes with a concentration of 0.5 ug/ml Doxorubicin (53% to 68% viable), see Figure 9. Pretreatment with a combination of 5 mM sodium pyruvate, 10 U Vitamin E, and 0.5% fatty acids prevented cytotoxity to peripheral blood monocytes with a concentration of 1 ug/ml Doxorubicin (47% to 69% viable), see Figure 13. Pretreatment with the single agent 50 U Vitamin E prevented cytotoxity to U937 tumor cells induced by 1 ug/ml Doxorubicin (42% to 62% viable), see Figure 7.

The viability of cultured peripheral monocytes without Doxorubicin was 66% and increased to 75% with the cytoprotective combination of 5 mM sodium pyruvate, 10 U Vitamin E, and 0.5% fatty acids, see Figure 13. The viability of cultured peripheral monocytes treated with 0.5 ug/ml Doxorubicin was 47% and increased to 63.5% when pretreated with the cytoprotective combination of 5 mM sodium pyruvate, 10 U Vitamin E, and 0.5% fatty acids,

see Figure 13. The viability of cultured peripheral monocytes treated with 1 ug/ml Doxorubicin was 42% and increased to 66% when pretreated with the cytoprotective combination of 5 mM sodium pyruvate, 10 U Vitamin E, and 0.5% fatty acids, see Figure 13.

The viability of cultured U937 tumor cells without Doxorubicin was 67% and did not increase when treated with any of the agents, see Figure 13. The viability of cultured U937 tumor cells with 0.5 ug/ml Doxorubicin treatment was 47% and the highest increase in viability occurred with pretreatment of 50 U Vitamin E and 0.5% fatty acids, see Figure 12. The viability of cultured U937 tumor cells with 1 ug/ml Doxorubicin treatment was 45% and the highest increase in viability occurred with pretreatment of 10 U Vitamin E and 0.5% fatty acids, see Figure 12.

Optimal concentrations of the cytoprotective agents to prevent Doxorubicin-induced cytotoxity were found to be 5 mM sodium pyruvate, 10-50 U Vitamin E, and 0.5% fatty acids. In wash-out studies, the cytoprotective combination of sodium pyruvate, Vitamin E, and fatty acids and the combination of 5mM sodium pyruvate and 10 U Vitamin E protected the normal peripheral blood monocytes from Doxorubicin-induced cytotoxity, see Figure 13. Vitamin E alone and fatty acids alone prevented the cytotoxity of Doxorubicin in U937 cells, see Figure 11. When normal peripheral blood monocytes were co-cultured with U937 monocytic leukemia tumor cells, the cytoprotective combination of 5 mM sodium pyruvate, 0.5% fatty acids, and 10 U Vitamin E provided significant protection to the normal peripheral monocytes from Doxorubicin-induced cytotoxity and did not protect the tumor cells from the effects of the cytotoxic agent, see Figure 24.

These results show that the combination of agents 5 mM sodium pyruvate, 0.5% fatty acids, and 10 U

and 50U Vitamin E are useful as selective cytoprotective agents for use with compounds that are toxic to normal cells as well as tumor cells.

The invention being thus described, it will be obvious that the same may be varied in many ways. Such variations are not to be regarded as a departure from the spirit and scope of the invention and all such modifications are intended to be included within the scope of the following claims.

I claim:

1. A cytoprotective composition for preventing and reducing injury to mammalian cells from a medicament having cytotoxic properties which comprises:

(a) pyruvate selected from the group consisting of pyruvic acid, pharmaceutically acceptable salts of pyruvic acid, and mixtures thereof; and

(b) an antioxidant.

2. The cytoprotective composition according to claim 1, wherein the pyruvate is selected from the group consisting of pyruvic acid, sodium pyruvate, potassium pyruvate, magnesium pyruvate, calcium pyruvate, zinc pyruvate, manganese pyruvate, and mixtures thereof.

3. The cytoprotective composition according to claim 2, wherein the pyruvate is sodium pyruvate.

4. The cytoprotective composition according to claim 1, wherein the antioxidant is selected from the group consisting of retinol, 3, 4-didehydroretinol, *alpha*-carotene, *beta*-carotene, *gamma*-carotene, *delta*-carotene, ascorbic acid, *alpha*-tocopherol, *beta*-tocopherol, *gamma*-tocopherol, *delta*-tocopherol, and mixtures thereof.

5. The cytoprotective composition according to claim 4, wherein the antioxidant is *alpha*-tocopherol.

6. The cytoprotective composition according to claim 1, wherein pyruvate is present in the cytoprotective composition in an amount from about 10% to about 75%, by weight.

7. The cytoprotective composition according to claim 1, wherein the antioxidant is present in the cytoprotective composition in an amount from about 10% to about 75%, by weight.

8. A cytoprotective composition for preventing and reducing injury to mammalian cells from a medicament having cytotoxic properties which comprises:

(a) pyruvate selected from the group consisting of pyruvic acid, pharmaceutically acceptable salts of pyruvic acid, and mixtures thereof;

(b) an antioxidant; and

(c) a mixture of saturated and unsaturated fatty acids wherein the fatty acids are those fatty acids required for the repair of cellular membranes and resuscitation of mammalian cells.

9. The cytoprotective composition according to claim 8, wherein the mammalian cells comprise epidermal keratinocytes.

10. The cytoprotective composition according to claim 8, wherein the pyruvate is selected from the group consisting of pyruvic acid, sodium pyruvate, potassium pyruvate, magnesium pyruvate, calcium pyruvate, zinc pyruvate, manganese pyruvate, and mixtures thereof.

11. The cytoprotective composition according to claim 8, wherein the antioxidant is selected from the group consisting of retinol, 3, 4-didehydroretinol, *alpha*-carotene, *beta*-carotene, *gamma*-carotene, *delta*-carotene, ascorbic acid, *alpha*-tocopherol, *beta*-tocopherol, *gamma*-tocopherol, *delta*-tocopherol, and mixtures thereof.

12. The cytoprotective composition according to claim 8, wherein the mixture of saturated and unsaturated fatty acids comprises animal and vegetable fats and waxes.

13. The cytoprotective composition according to claim 12, wherein the mixture of saturated and unsaturated fatty acids comprises human fat, chicken fat, cow fat, sheep fat, horse fat, pig fat, and whale fat.

14. The cytoprotective composition according to claim 13, wherein the mixture of saturated and unsaturated fatty acids comprises lauric acid, myristic acid, myristoleic acid, pentadecanoic acid, palmitic acid, palmitoleic acid, margaric acid, margaroleic acid, stearic, oleic acid, linoleic acid, linolenic acid, arachidic acid, and gaddoleic acid.

15. The cytoprotective composition according to claim 8, wherein pyruvate is present in the cytoprotective composition in an amount from about 10% to about 50%, by weight.

16. The cytoprotective composition according to claim 8, wherein the antioxidant is present in the cytoprotective composition in an amount from about 10% to about 50%, by weight.

17. The cytoprotective composition according to claim 8, wherein the mixture of saturated and unsaturated fatty acids is present in the cytoprotective composition in an amount from about 10% to about 50%, by weight.

18. A cytoprotective pharmaceutical composition for preventing and reducing injury to mammalian cells from a medicament having cytotoxic properties which comprises:

(A) a cytoprotective composition selected from the group consisting of:

(1) (a) pyruvate selected from the group consisting of pyruvic acid, pharmaceutically acceptable salts of pyruvic acid, and mixtures thereof;

(b) an antioxidant; and

(c) a mixture of saturated and unsaturated fatty acids wherein the fatty acids are those fatty acids required for the resuscitation of injured mammalian cells; and

(2) (a) pyruvate selected from the group consisting of pyruvic acid, pharmaceutically acceptable salts of pyruvic acid, and mixtures thereof; and

(b) an antioxidant; and

(B) a medicament having cytotoxic properties.

19. A timed-release cytoprotective pharmaceutical composition for selectively protecting non-cancerous mammalian cells in the presence of cancerous mammalian cells from an anticancer medicament having cytotoxic properties which comprises:

(A) a cytoprotective composition in immediate release form selected from the group consisting of:

(1) (a) pyruvate selected from the group consisting of pyruvic acid, pharmaceutically acceptable salts of pyruvic acid, and mixtures thereof;

(b) an antioxidant; and

(c) a mixture of saturated and unsaturated fatty acids wherein the fatty acids are those fatty acids required for the resuscitation of injured mammalian cells; and

(2) (a) pyruvate selected from the group consisting of pyruvic acid, pharmaceutically acceptable salts of pyruvic acid, and mixtures thereof; and

(b) an antioxidant; and

(B) an anticancer medicament having cytotoxic properties in timed-release form;

wherein the cytoprotective composition is released substantially immediately and the anticancer medicament is released after a period of time sufficient such that the cancerous cells have substantially metabolized the cytoprotective composition and the non-cancerous cells have not substantially metabolized the cytoprotective composition.

20. A method for preparing a cytoprotective composition for preventing and reducing injury to mammalian cells from a medicament having cytotoxic properties which comprises the steps of admixing the following ingredients:

(a) pyruvate selected from the group consisting of pyruvic acid, pharmaceutically acceptable salts of pyruvic acid, and mixtures thereof; and

(b) an antioxidant.

21. A method for preparing a cytoprotective composition for preventing and reducing injury to mammalian cells from a medicament having cytotoxic properties which comprises the steps of admixing the following ingredients:

(a) pyruvate selected from the group consisting of pyruvic acid, pharmaceutically acceptable salts of pyruvic acid, and mixtures thereof;

(b) an antioxidant; and

(c) a mixture of saturated and unsaturated fatty acids wherein the fatty acids are those fatty acids required for the repair of cellular membranes and resuscitation of mammalian cells.

22. A method for preparing a cytoprotective pharmaceutical composition for preventing and reducing injury to mammalian cells from a medicament having cytotoxic properties which comprises the steps of:

(A) providing a cytoprotective composition selected from the group consisting of:

(1) (a) pyruvate selected from the group consisting of pyruvic acid, pharmaceutically acceptable salts of pyruvic acid, and mixtures thereof;

(b) an antioxidant; and

(c) a mixture of saturated and unsaturated fatty acids wherein the fatty acids are those fatty acids required for the resuscitation of injured mammalian cells; and

(2) (a) pyruvate selected from the group consisting of pyruvic acid, pharmaceutically acceptable salts of pyruvic acid, and mixtures thereof; and

(b) an antioxidant; and

(B) providing an anticancer medicament having cytotoxic properties; and

(C) admixing the cytoprotective composition from step (A) with the medicament from step (B) to prepare the cytoprotective pharmaceutical composition.

23. A method for preparing a timed-release cytoprotective pharmaceutical composition for selectively protecting non-cancerous mammalian cells in the presence of cancerous mammalian cells from an anticancer medicament having cytotoxic properties which comprises the steps of:

(A) providing a cytoprotective composition in an immediate release form selected from the group consisting of:

(1) (a) pyruvate selected from the group consisting of pyruvic acid, pharmaceutically acceptable salts of pyruvic acid, and mixtures thereof;

(b) an antioxidant; and

(c) a mixture of saturated and unsaturated fatty acids wherein the fatty acids are those fatty acids required for the resuscitation of injured mammalian cells; and

(2) (a) pyruvate selected from the group consisting of pyruvic acid, pharmaceutically acceptable salts of pyruvic acid, and mixtures thereof; and

(b) an antioxidant; and

(B) providing an anticancer medicament having cytotoxic properties in timed-release form; and

(C) admixing the cytoprotective composition from step (A) with the medicament from step (B) to prepare the timed-release cytoprotective pharmaceutical composition;

wherein the cytoprotective composition is released substantially immediately and the anticancer medicament is released after a period of time sufficient such that the cancerous cells have substantially metabolized the cytoprotective composition and the non-cancerous cells have not substantially metabolized the cytoprotective composition.

24. A method for protecting mammalian cells from a medicament having cytotoxic properties which comprises the steps of:

(A) providing a cytoprotective composition selected from the group consisting of:

(1) (a) pyruvate selected from the group consisting of pyruvic acid, pharmaceutically acceptable salts of pyruvic acid, and mixtures thereof;

(b) an antioxidant; and

(c) a mixture of saturated and unsaturated fatty acids wherein the fatty acids are those fatty acids required for the resuscitation of injured mammalian cells; and

(2) (a) pyruvate selected from the group consisting of pyruvic acid, pharmaceutically acceptable salts of pyruvic acid, and mixtures thereof; and

(b) an antioxidant; and

(B) providing an anticancer medicament having cytotoxic properties; and

(C) administering the cytoprotective composition from step (A) and the medicament from step (B) concurrently to mammalian cells to protect the mammalian cells from the medicament having cytotoxic properties.

25. A method for selectively protecting non-cancerous mammalian cells in the presence of cancerous mammalian cells from an anticancer medicament having cytotoxic properties which comprises the steps of:

(A) providing a cytoprotective composition in an immediate release form selected from the group consisting of:

(1) (a) pyruvate selected from the group consisting of pyruvic acid, pharmaceutically acceptable salts of pyruvic acid, and mixtures thereof;

(b) an antioxidant; and

(c) a mixture of saturated and unsaturated fatty acids wherein the fatty acids are those fatty acids required for the resuscitation of injured mammalian cells; and

(2) (a) pyruvate selected from the group consisting of pyruvic acid, pharmaceutically acceptable salts of pyruvic acid, and mixtures thereof; and

(b) an antioxidant; and

(B) providing an anticancer medicament having cytotoxic properties in timed-release form; and

(C) administering the cytoprotective composition from step (A) and the medicament from step (B) concurrently to mammalian cells to selectively protect non-cancerous mammalian cells in the presence of cancerous mammalian cells from the anticancer medicament having cytotoxic properties;

wherein the cytoprotective composition is released substantially immediately and the anticancer medicament is released after a period of time sufficient such that the cancerous cells have substantially metabolized the cytoprotective composition and the non-cancerous cells have not substantially metabolized the cytoprotective composition.

26. A method for selectively protecting non-cancerous mammalian cells in the presence of cancerous mammalian cells from an anticancer medicament having cytotoxic properties which comprises the steps of:

(A) administering to mammalian cells a cytoprotective composition to prevent and reduce injury to the mammalian cells selected from the group consisting of:

(1) (a) pyruvate selected from the group consisting of pyruvic acid, pharmaceutically acceptable salts of pyruvic acid, and mixtures thereof;

(b) an antioxidant; and

(c) a mixture of saturated and unsaturated fatty acids wherein the fatty acids are those fatty acids required for the resuscitation of injured mammalian cells; and

(2) (a) pyruvate selected from the group consisting of pyruvic acid, pharmaceutically acceptable salts of pyruvic acid, and mixtures thereof; and

(b) an antioxidant; and

(B) waiting a period of time sufficient such that the cancerous cells have substantially metabolized the cytoprotective composition and the non-cancerous cells have not substantially metabolized the cytoprotective composition; and

(C) administering the cytotoxic anticancer medicament to the mammalian cells to treat the cancerous cells which are unprotected by the cytoprotective composition and the non-cancerous cells which are protected by the cytoprotective composition to thereby increase the therapeutic effect of the anticancer medicament.

27. A cytoprotective pharmaceutical composition which comprises a pharmaceutically acceptable carrier and a therapeutically effective amount of a cytoprotective composition for preventing and reducing injury to mammalian cells from a medicament having cytotoxic properties, wherein the cytoprotective composition is selected from the group consisting of:

(1) (a) pyruvate selected from the group consisting of pyruvic acid, pharmaceutically acceptable salts of pyruvic acid, and mixtures thereof;

(b) an antioxidant; and

(c) a mixture of saturated and unsaturated fatty acids wherein the fatty acids are those fatty acids required for the resuscitation of injured mammalian cells; and

(2) (a) pyruvate selected from the group consisting of pyruvic acid, pharmaceutically acceptable salts of pyruvic acid, and mixtures thereof; and

(b) an antioxidant.

28. The cytoprotective pharmaceutical composition according to claim 27, further comprising a medicament having cytotoxic properties.

29. The cytoprotective pharmaceutical composition according to claim 28, wherein the cytoprotective composition is in immediate release form and the medicament having cytotoxic properties is in sustained release form.

FIG. 1A

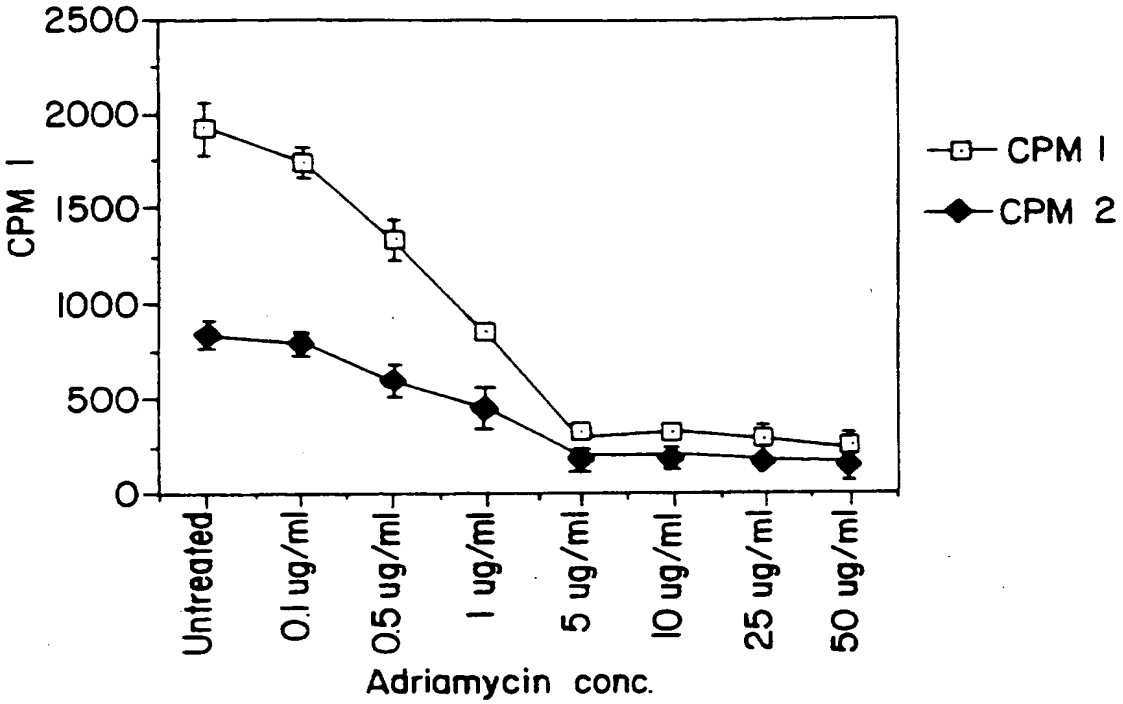


FIG. 1B

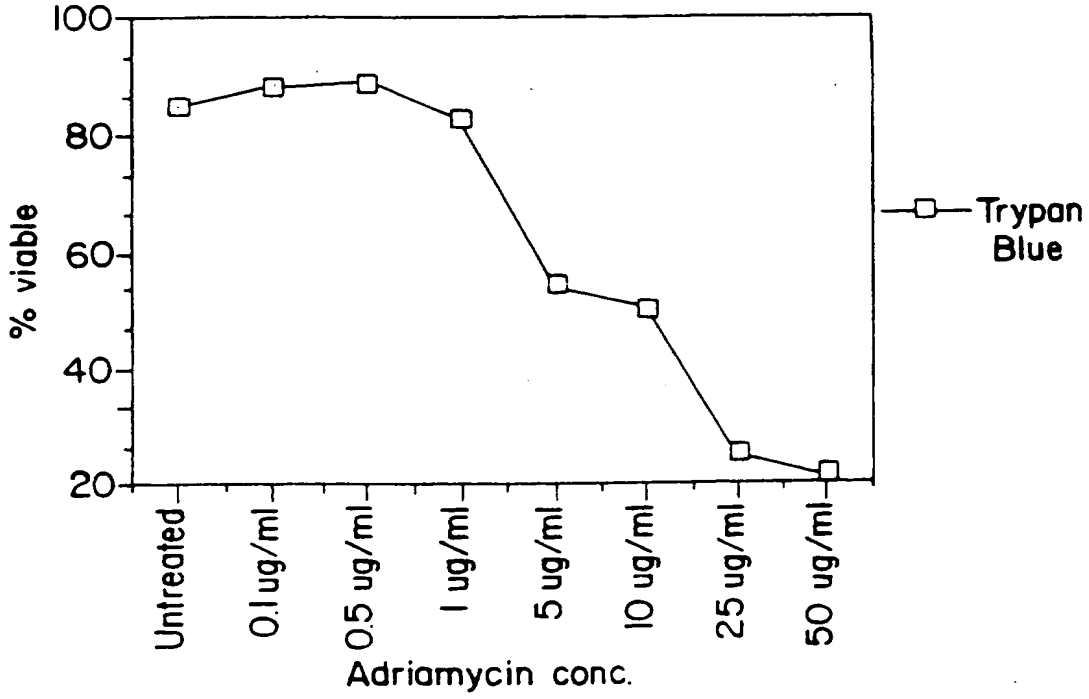


FIG. 2

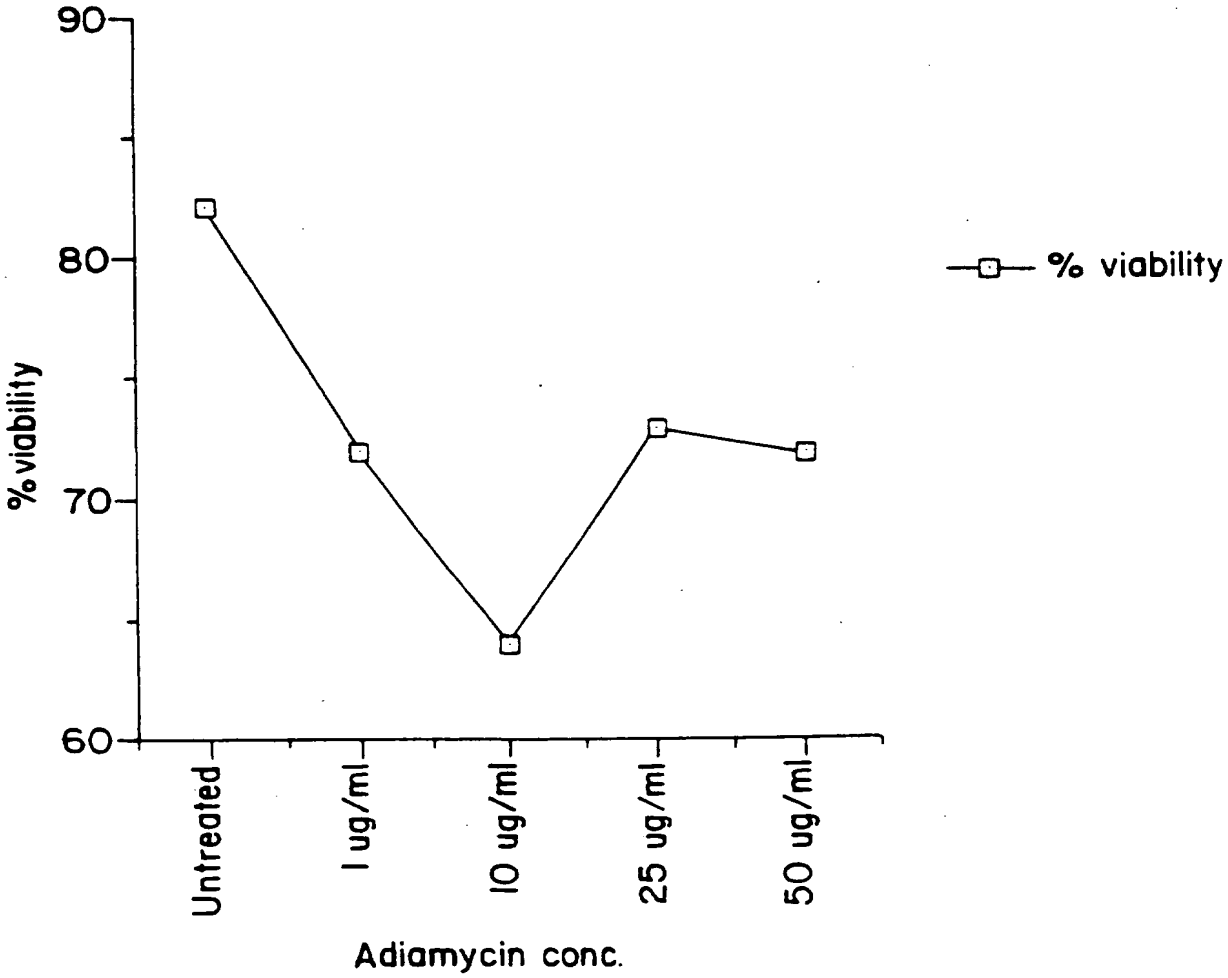

90
80
70
60
% viability
Untreated
1 ug/ml
10 ug/ml
25 ug/ml
50 ug/ml
Adiamycin conc.
% viability

FIG. 3

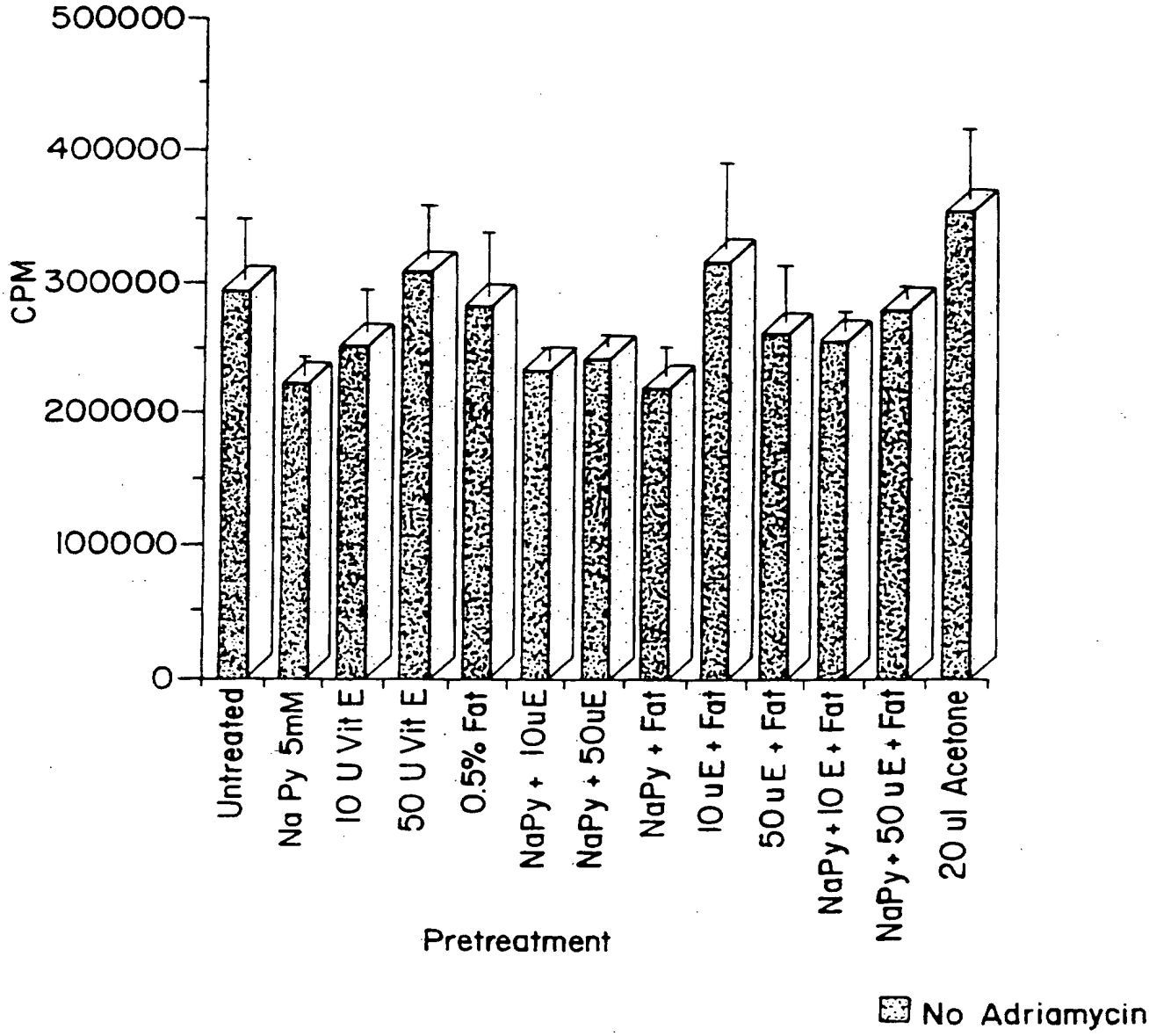

500000
400000
300000
200000
100000
0
CPM
Untreated
Na Py 5mM
10 U Vit E
50 U Vit E
0.5% Fat
NaPy + 10uE
NaPy + 50uE
NaPy + Fat
10 uE + Fat
50 uE + Fat
NaPy + 10 E + Fat
NaPy + 50 uE + Fat
20 ul Acetone
Pretreatment
No Adriamycin

FIG. 4A

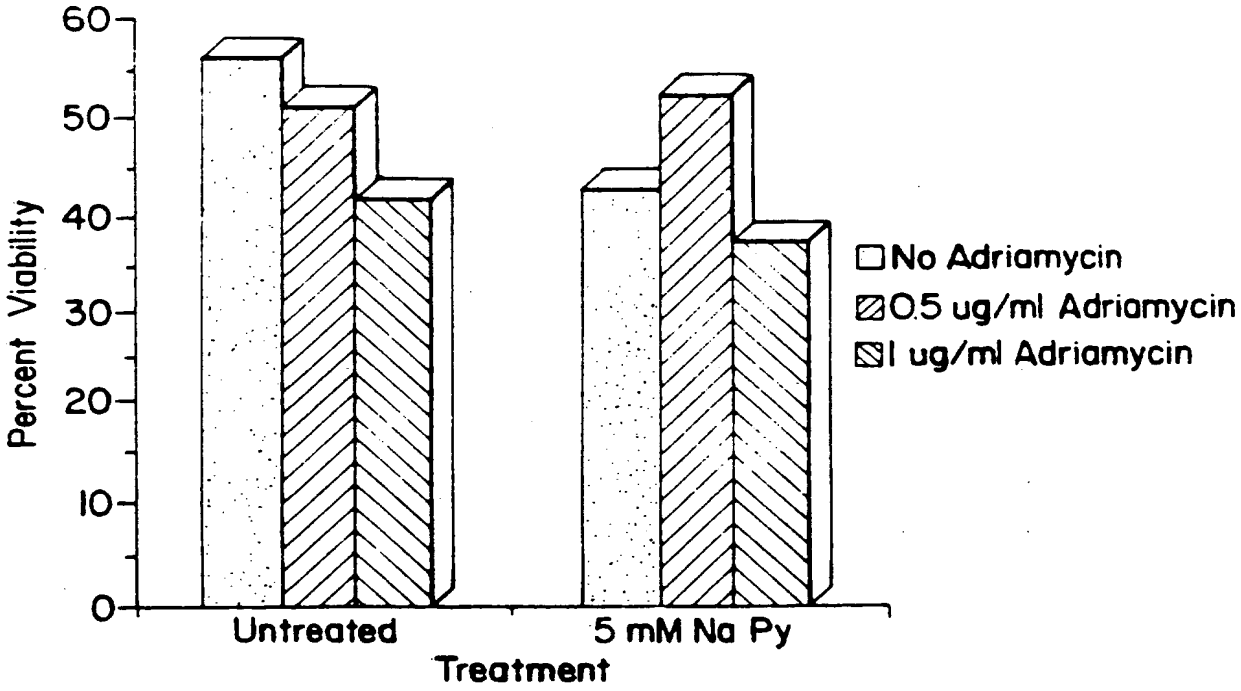

60
50
40
30
20
10
0
Percent Viability
Untreated
5 mM Na Py
Treatment
No Adriamycin
0.5 ug/ml Adriamycin
1 ug/ml Adriamycin

FIG. 4B

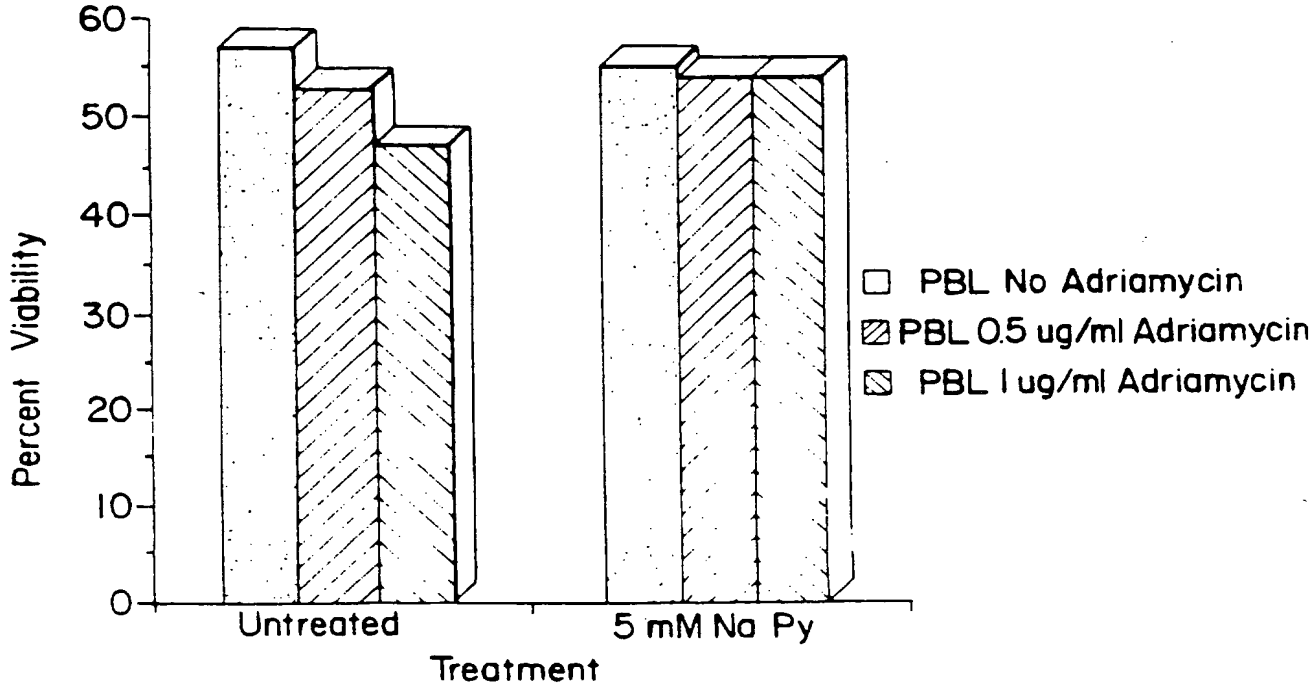

60
50
40
30
20
10
0
Percent Viability
Untreated
5 mM Na Py
Treatment
PBL No Adriamycin
PBL 0.5 ug/ml Adriamycin
PBL 1 ug/ml Adriamycin

FIG. 5A

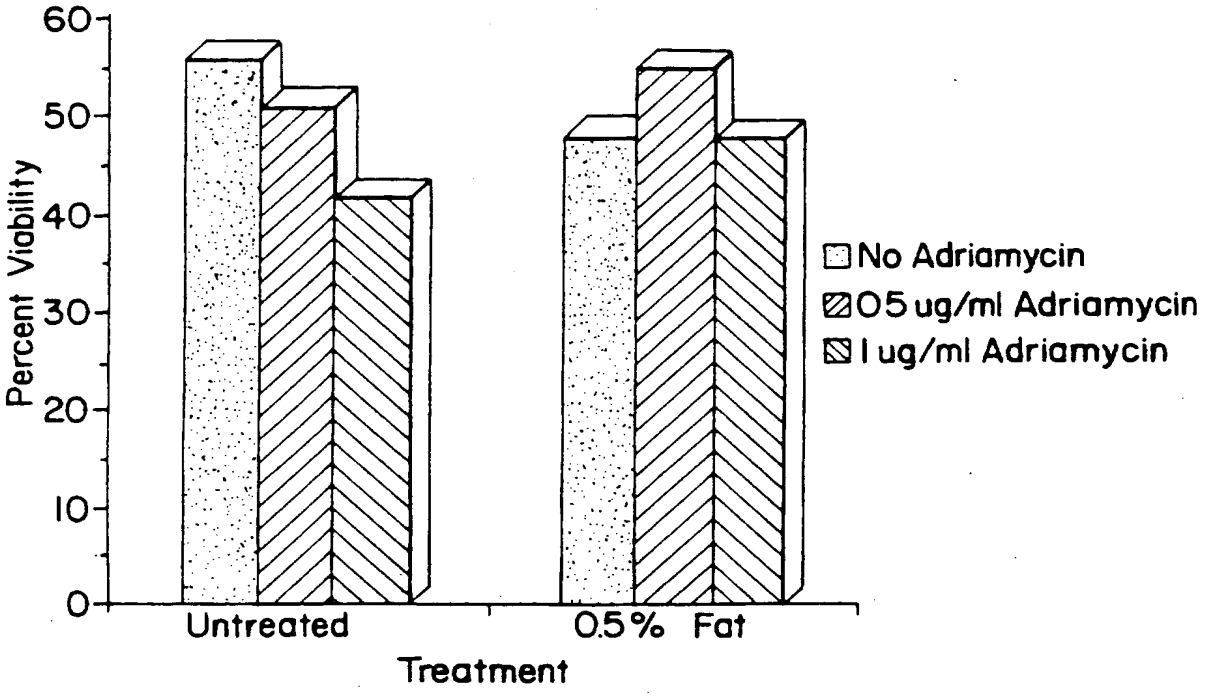


FIG. 5B

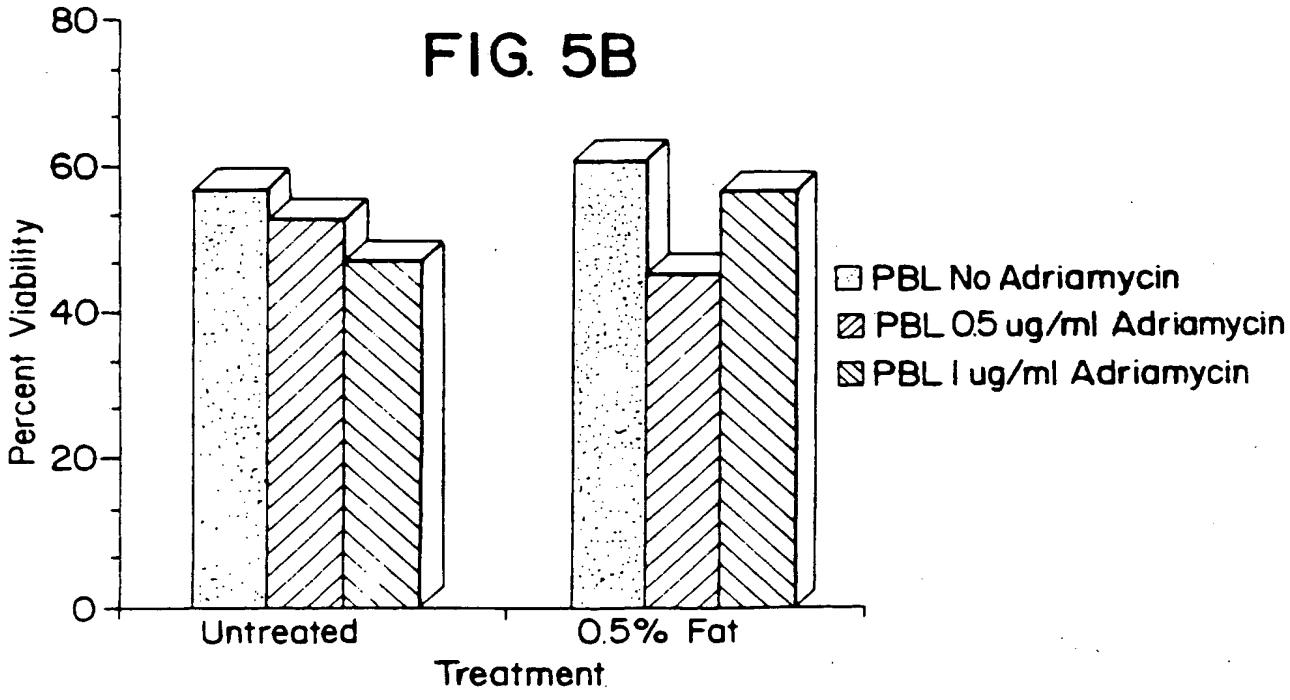


FIG. 6A

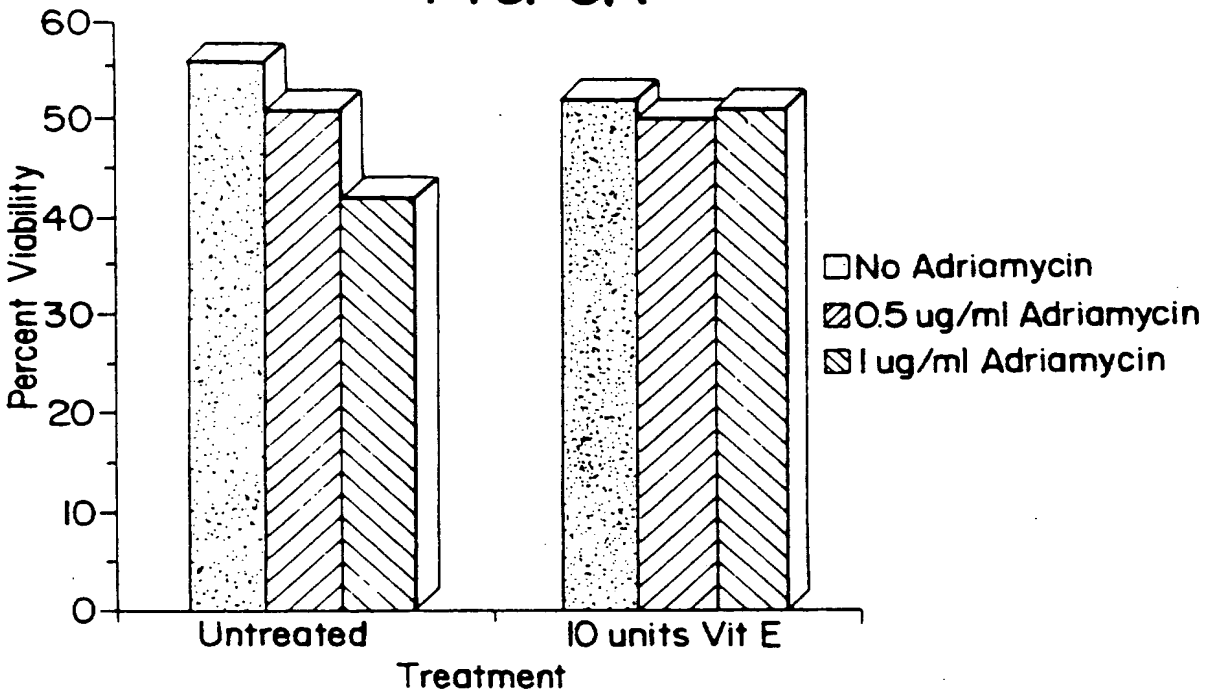

60
50
40
30
20
10
0
Percent Viability
Untreated
10 units Vit E
Treatment
No Adriamycin
0.5 ug/ml Adriamycin
1 ug/ml Adriamycin

FIG. 6B

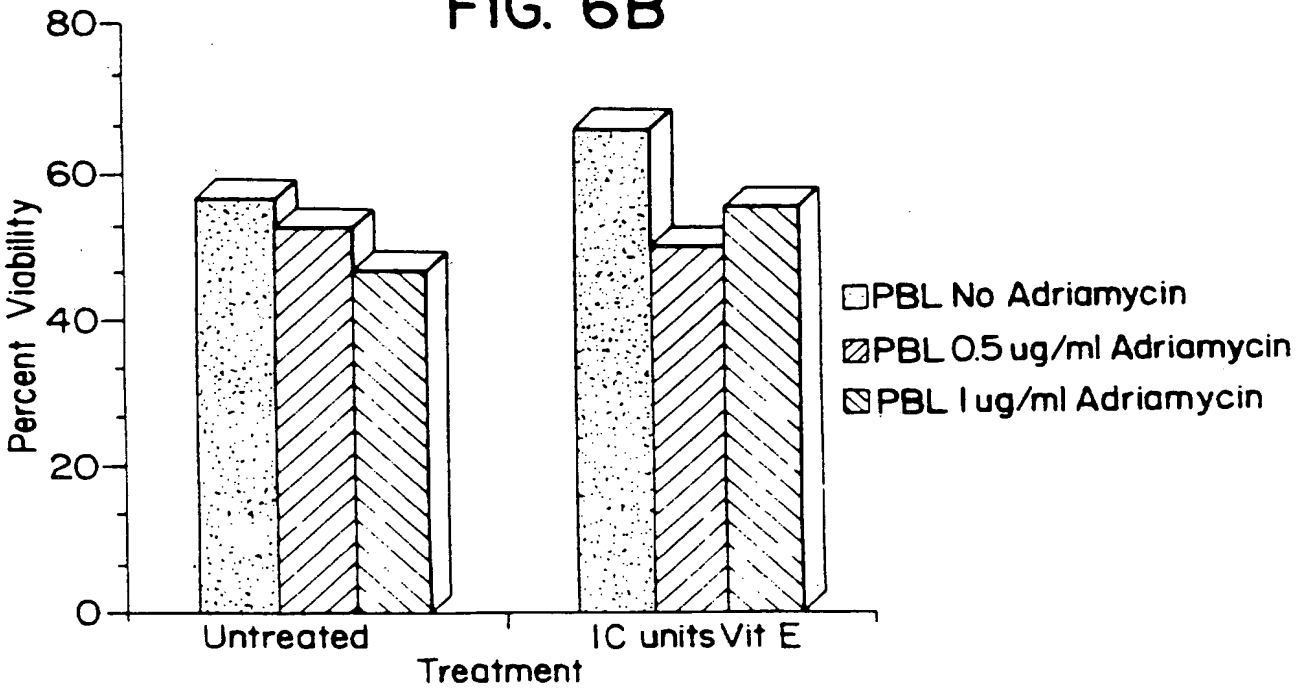

80
60
40
20
0
Percent Viability
Untreated
1C units Vit E
Treatment
PBL No Adriamycin
PBL 0.5 ug/ml Adriamycin
PBL 1 ug/ml Adriamycin

FIG. 7A

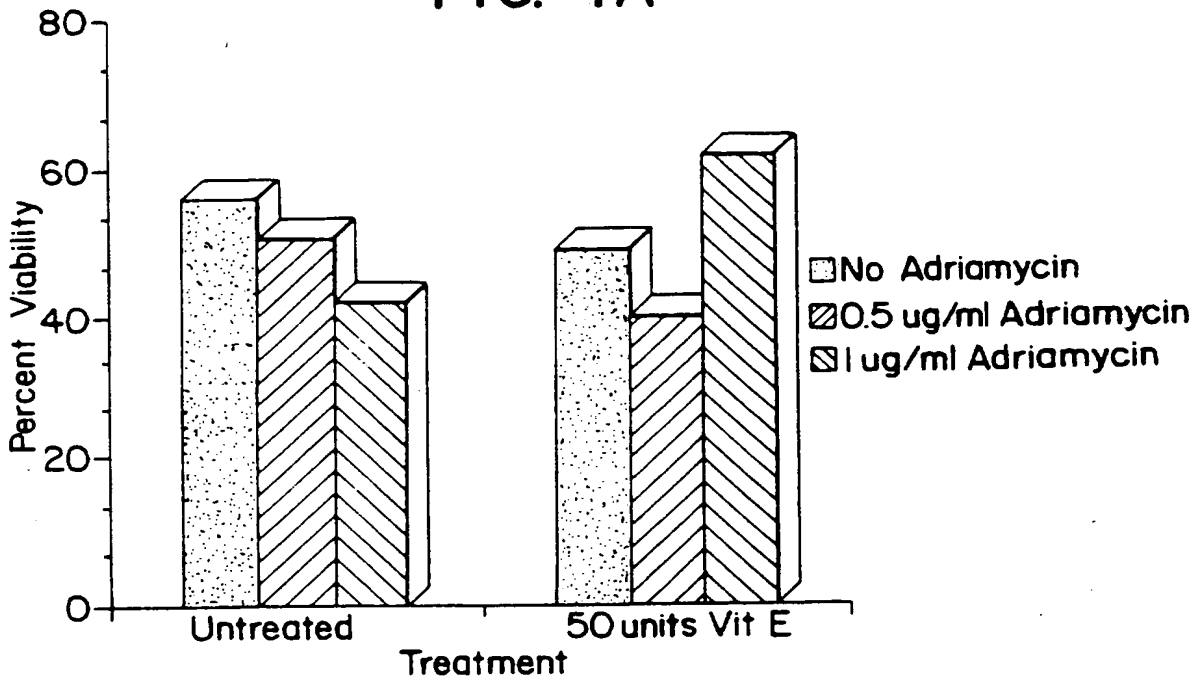

80
60
40
20
0
Percent Viability
Untreated
50 units Vit E
Treatment
No Adriamycin
0.5 ug/ml Adriamycin
1 ug/ml Adriamycin

FIG. 7B

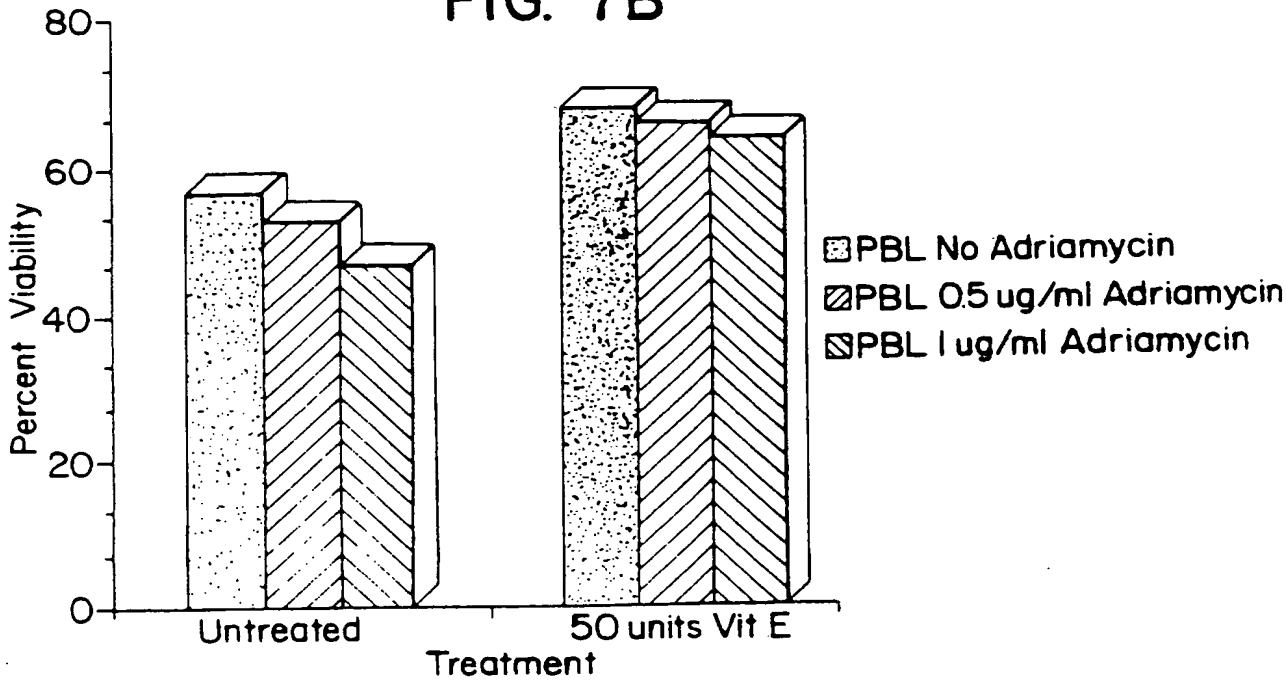

80
60
40
20
0
Percent Viability
Untreated
50 units Vit E
Treatment
PBL No Adriamycin
PBL 0.5 ug/ml Adriamycin
PBL 1 ug/ml Adriamycin

FIG. 8A

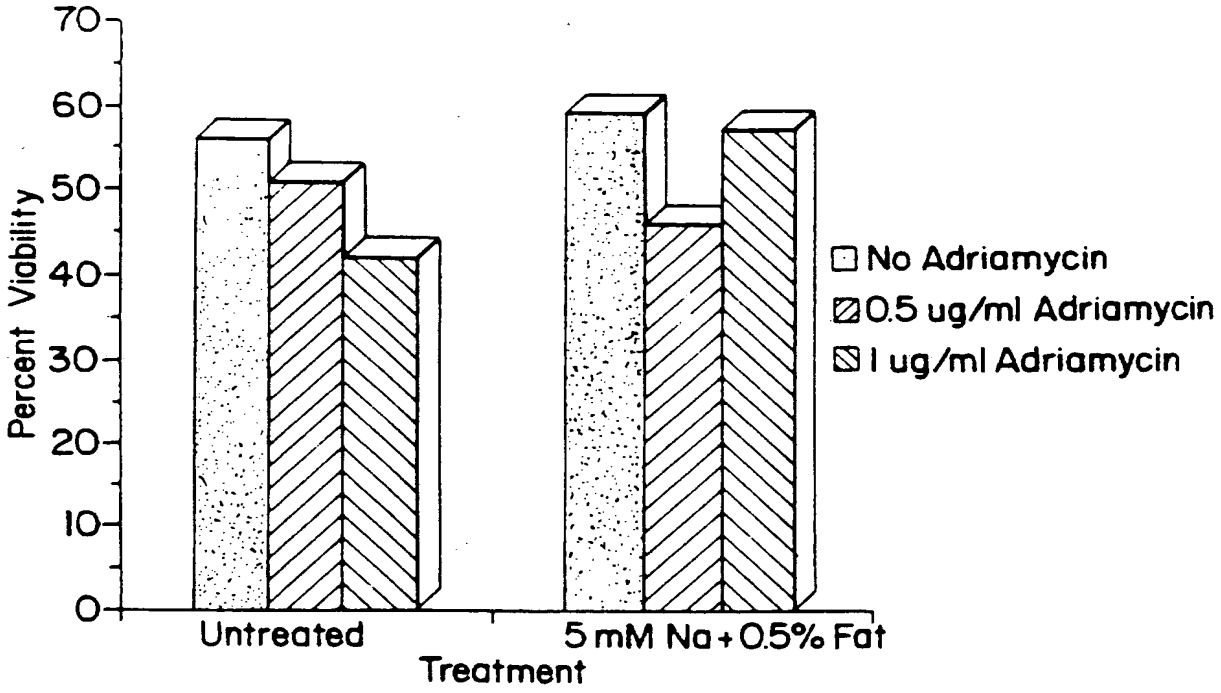

70
60
50
40
30
20
10
0
Percent Viability
Untreated
5 mM Na + 0.5% Fat
Treatment
No Adriamycin
0.5 ug/ml Adriamycin
1 ug/ml Adriamycin

FIG. 8B

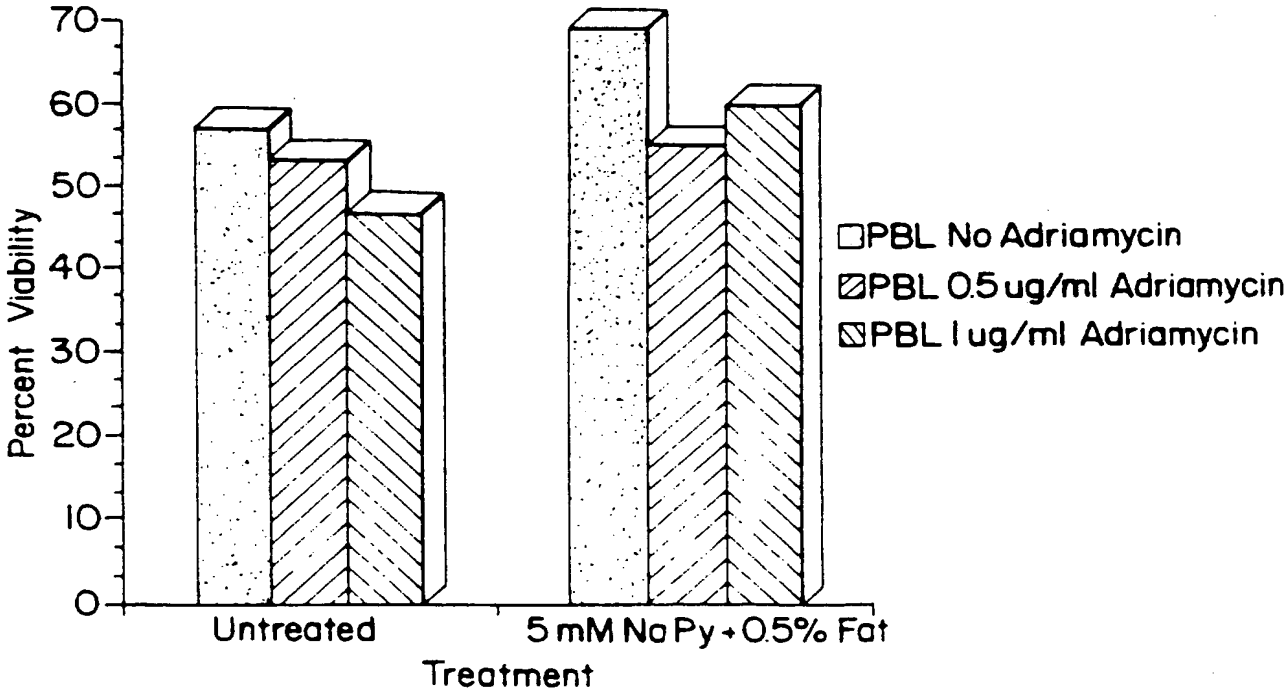

70
60
50
40
30
20
10
0
Percent Viability
Untreated
5 mM Na Py + 0.5% Fat
Treatment
PBL No Adriamycin
PBL 0.5 ug/ml Adriamycin
PBL 1 ug/ml Adriamycin

FIG. 9A

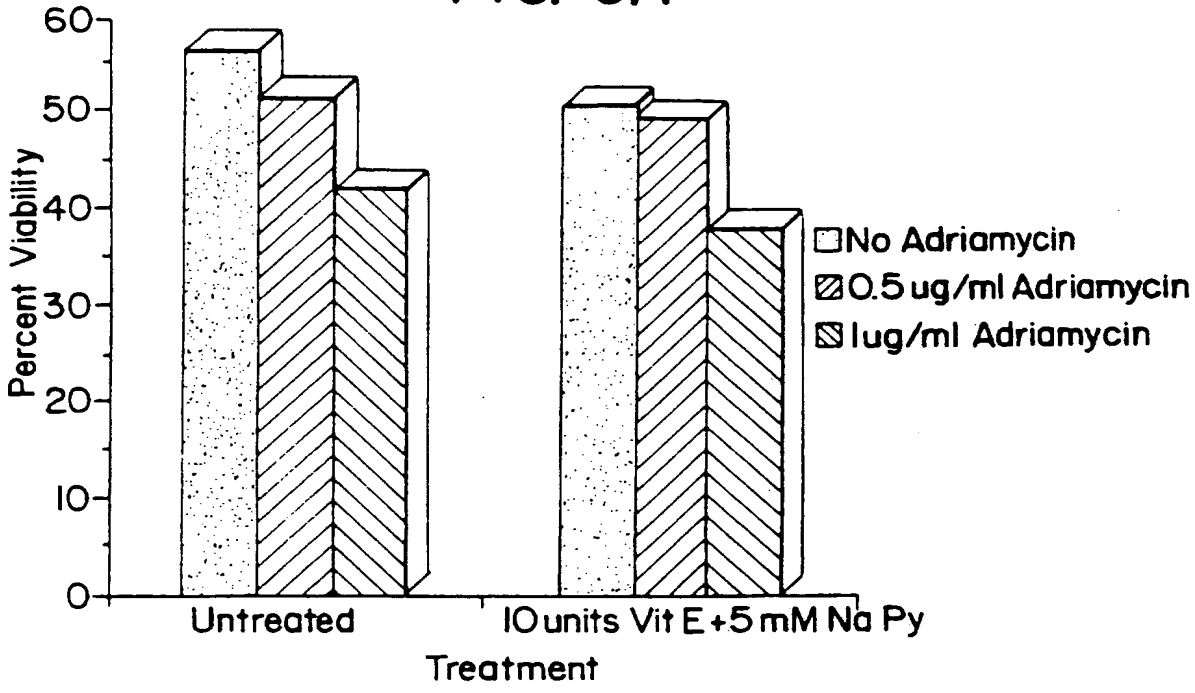

60
50
40
30
20
10
0
Percent Viability
No Adriamycin
0.5 ug/ml Adriamycin
1ug/ml Adriamycin
Untreated
10 units Vit E+5 mM Na Py
Treatment

FIG. 9B

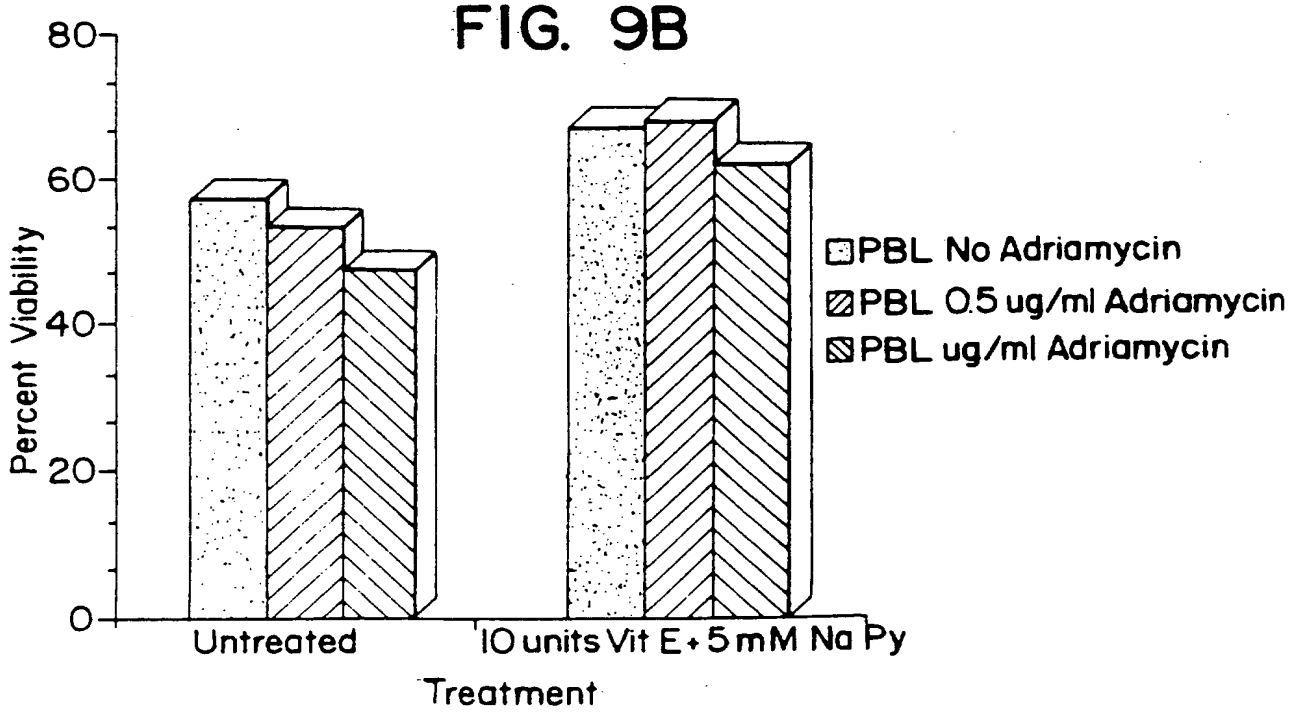

80
60
40
20
0
Percent Viability
PBL No Adriamycin
PBL 0.5 ug/ml Adriamycin
PBL ug/ml Adriamycin
Untreated
10 units Vit E+5 mM Na Py
Treatment

FIG. 10A

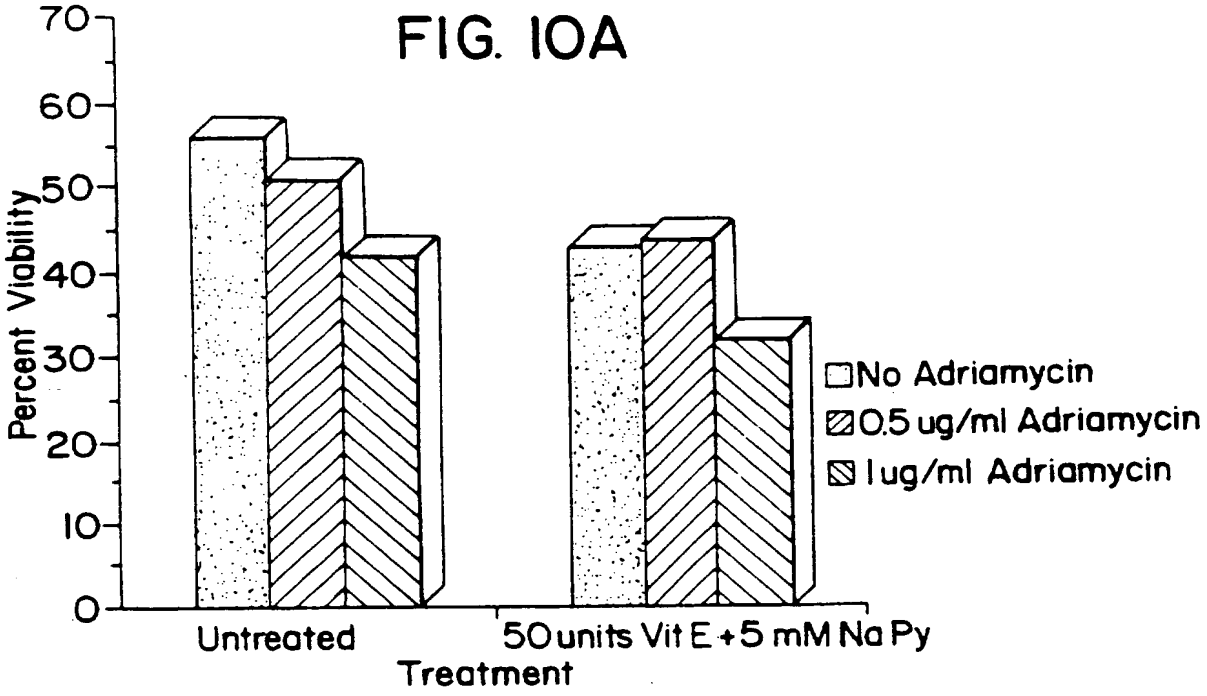


FIG. 10B

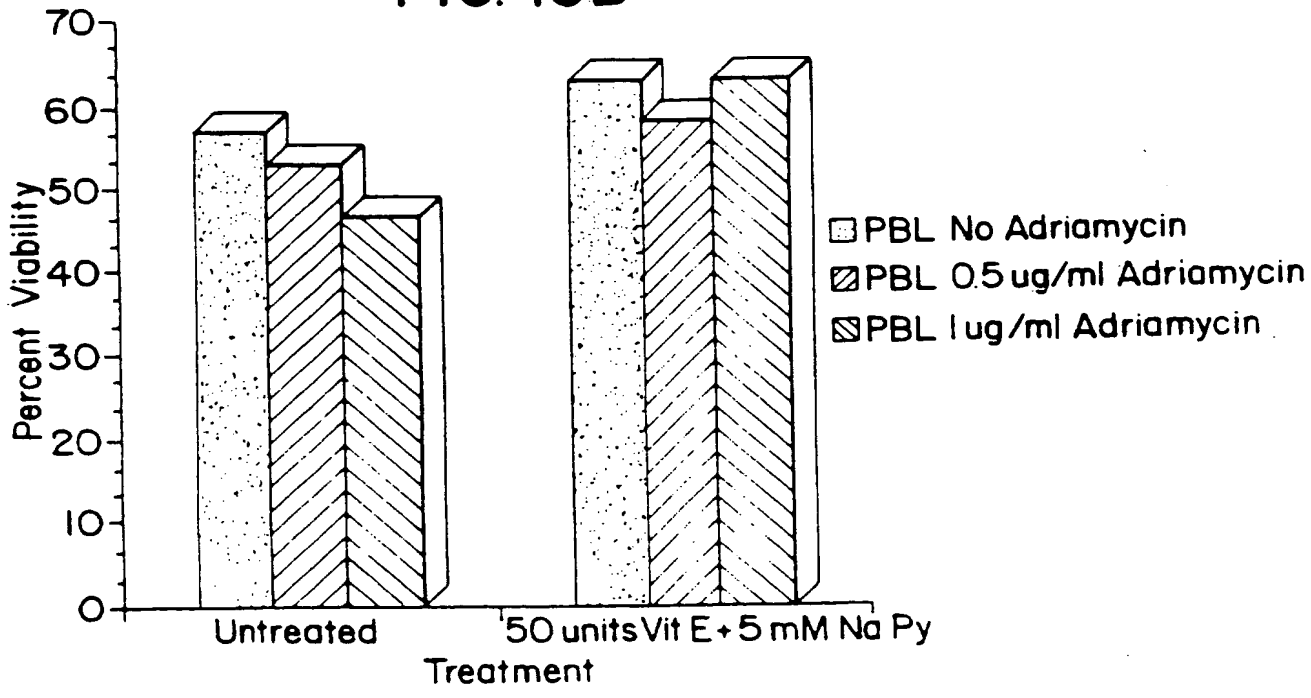


FIG. IIA

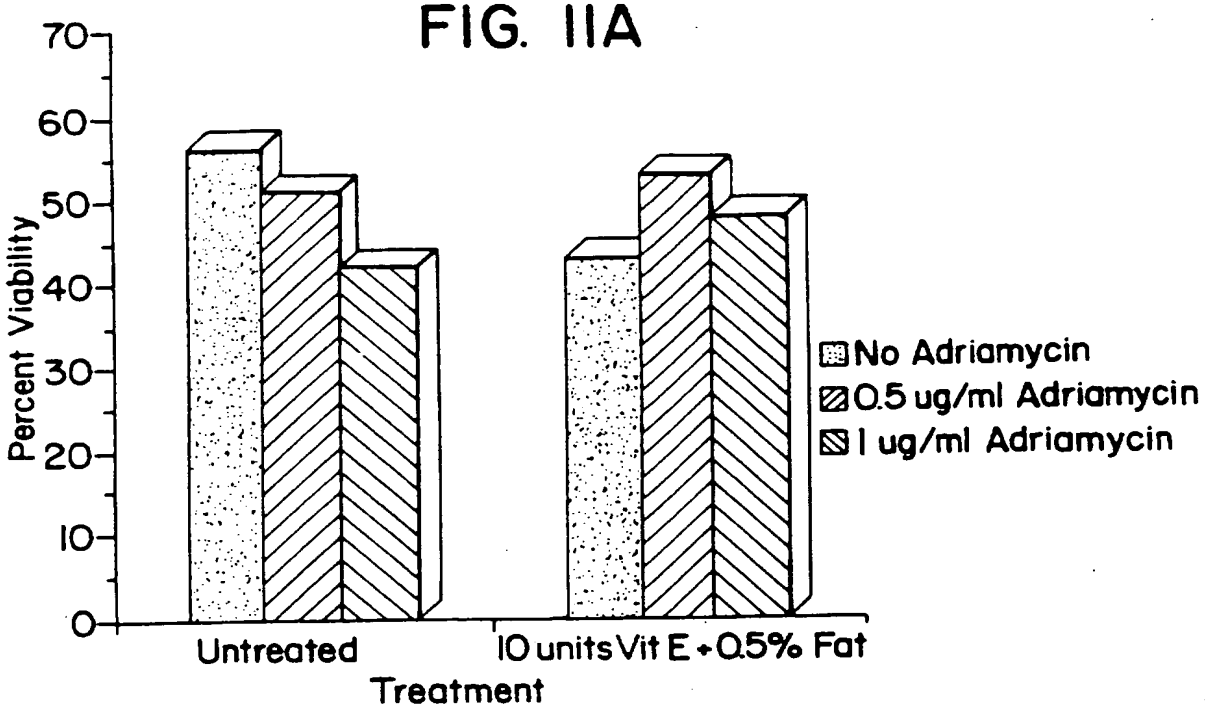


FIG. IIB

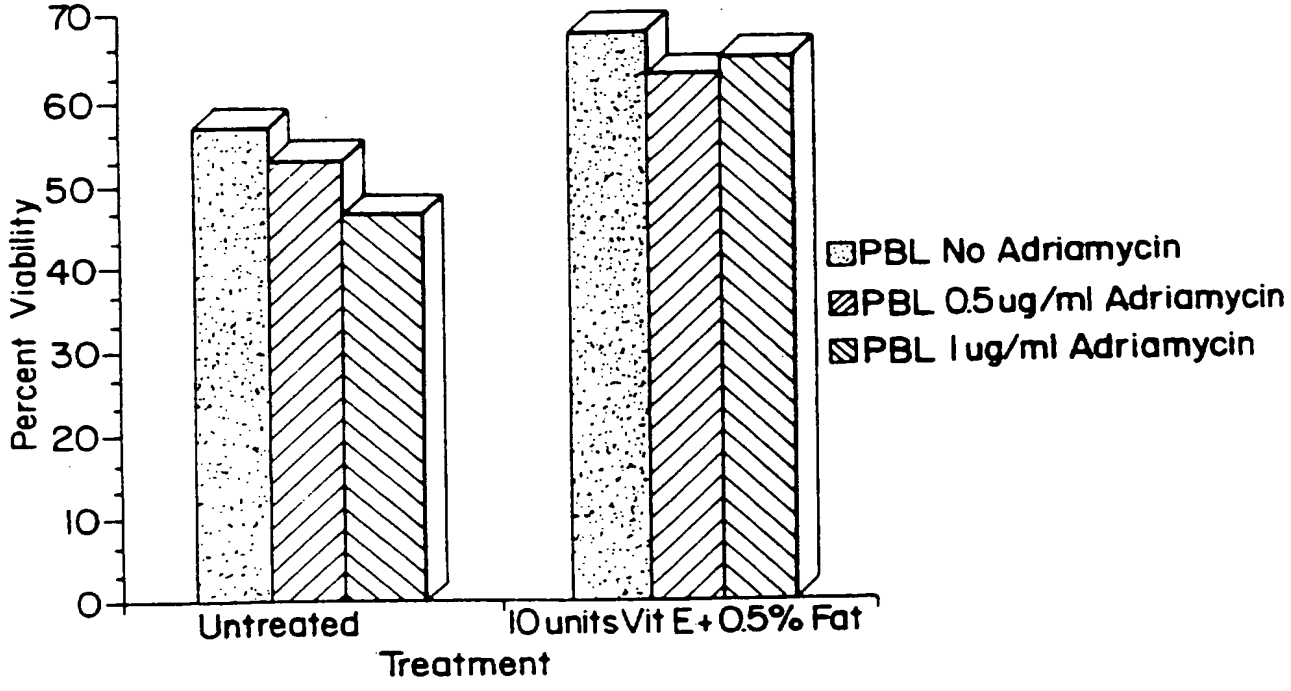


FIG. 12A

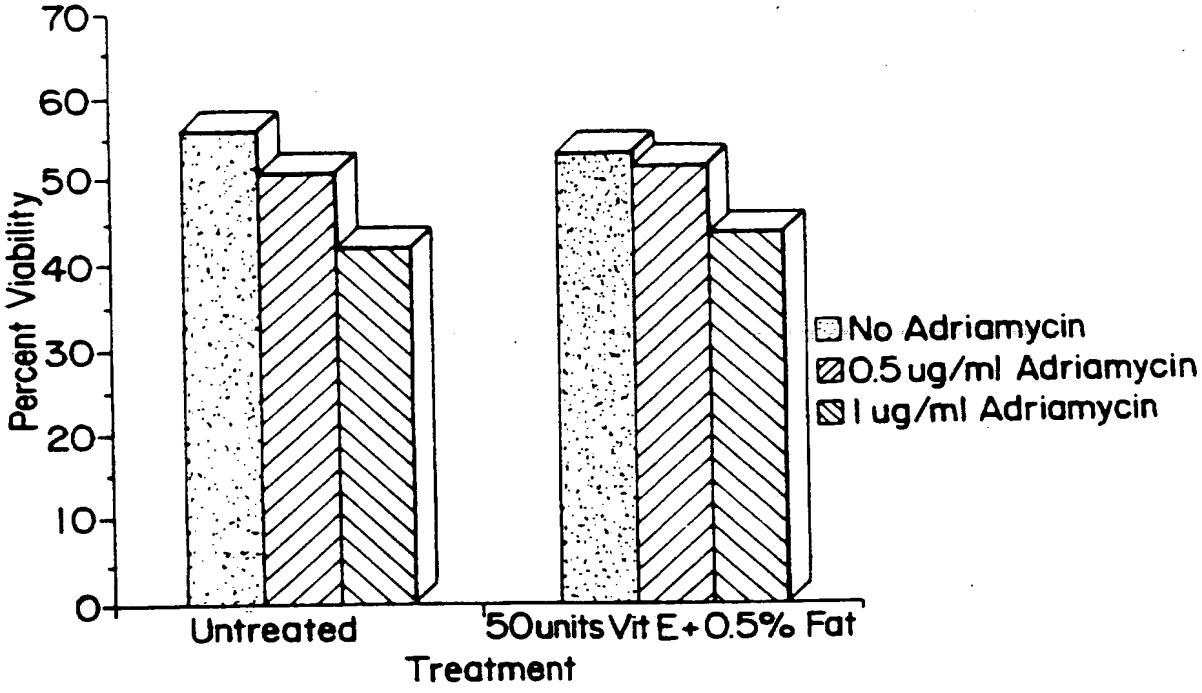


FIG. 12B

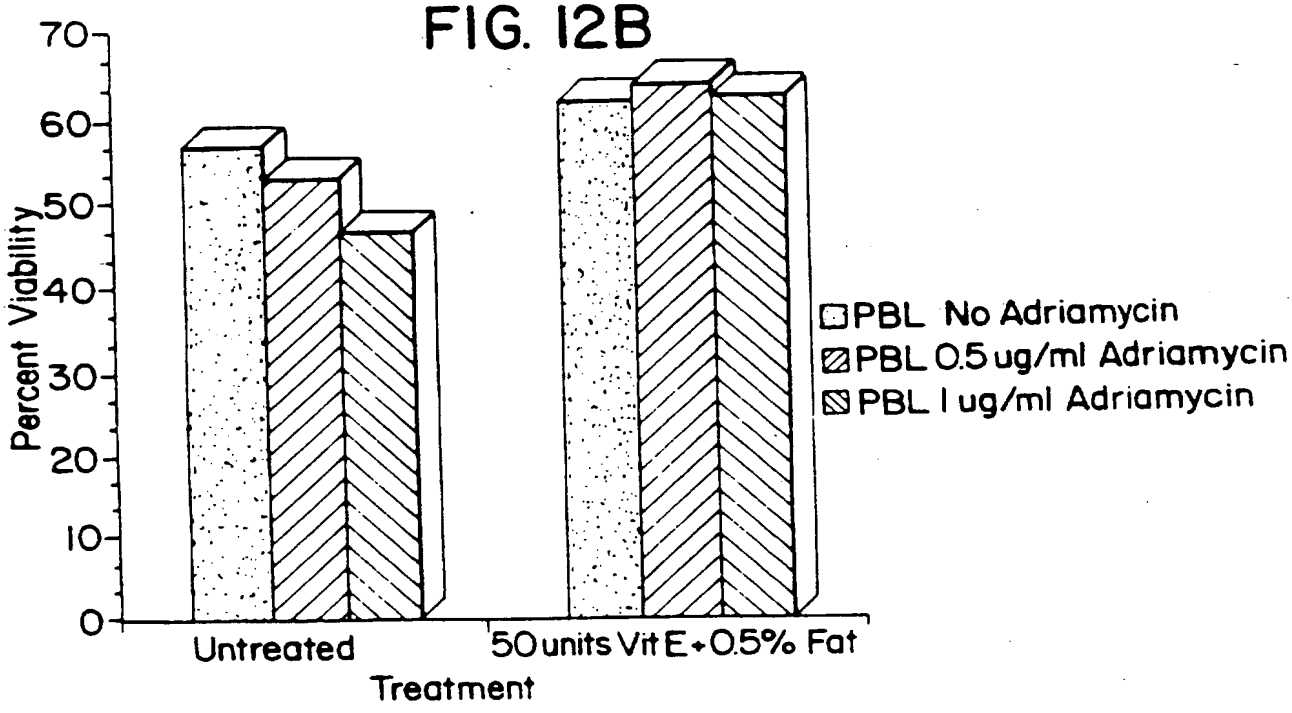


FIG. 13A

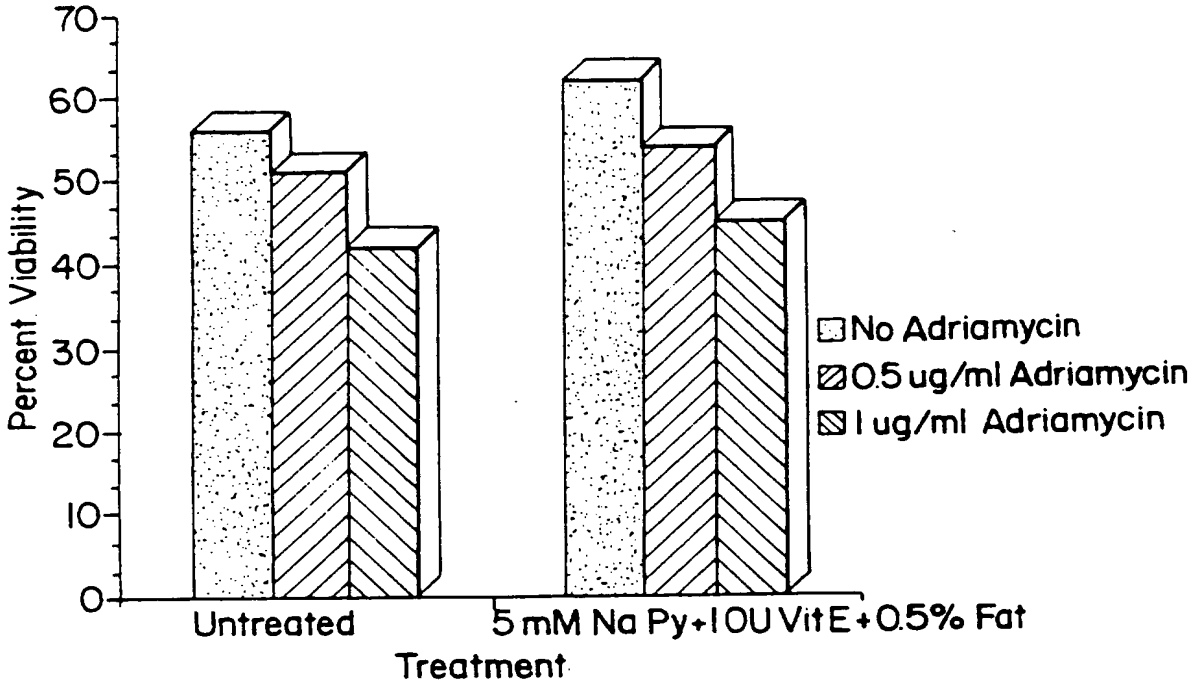


FIG. 13B

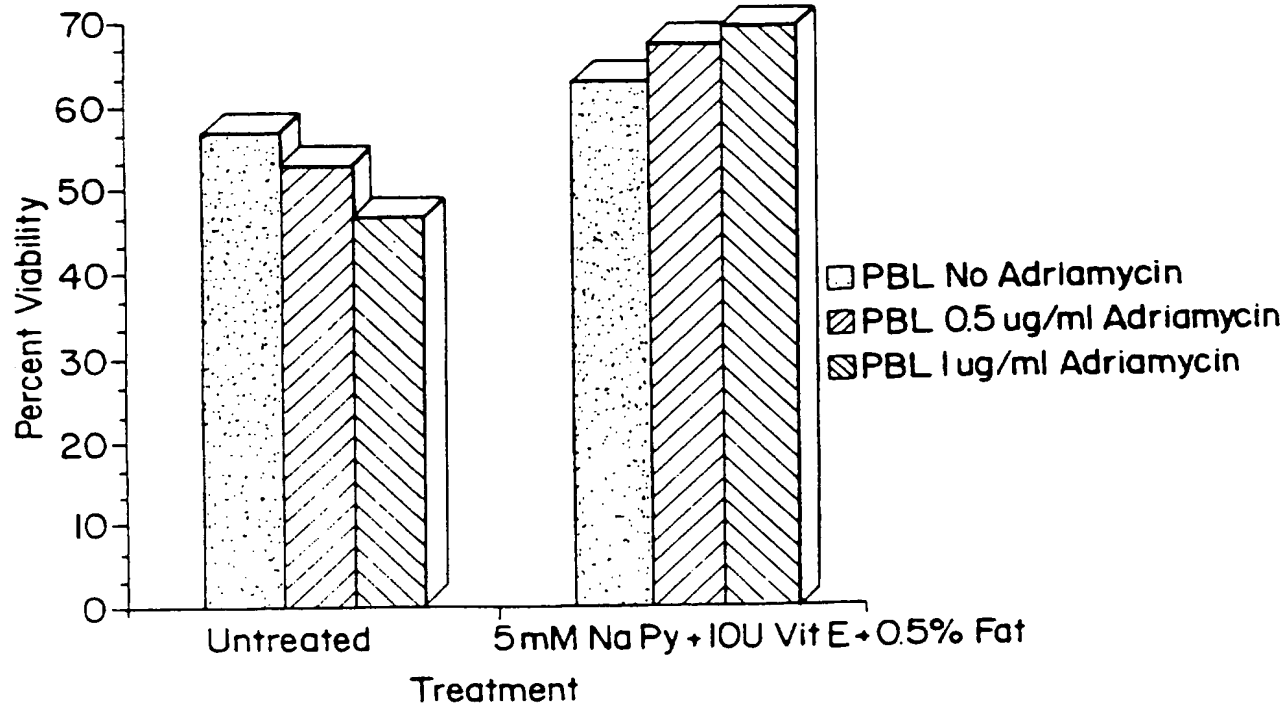


FIG. 14A

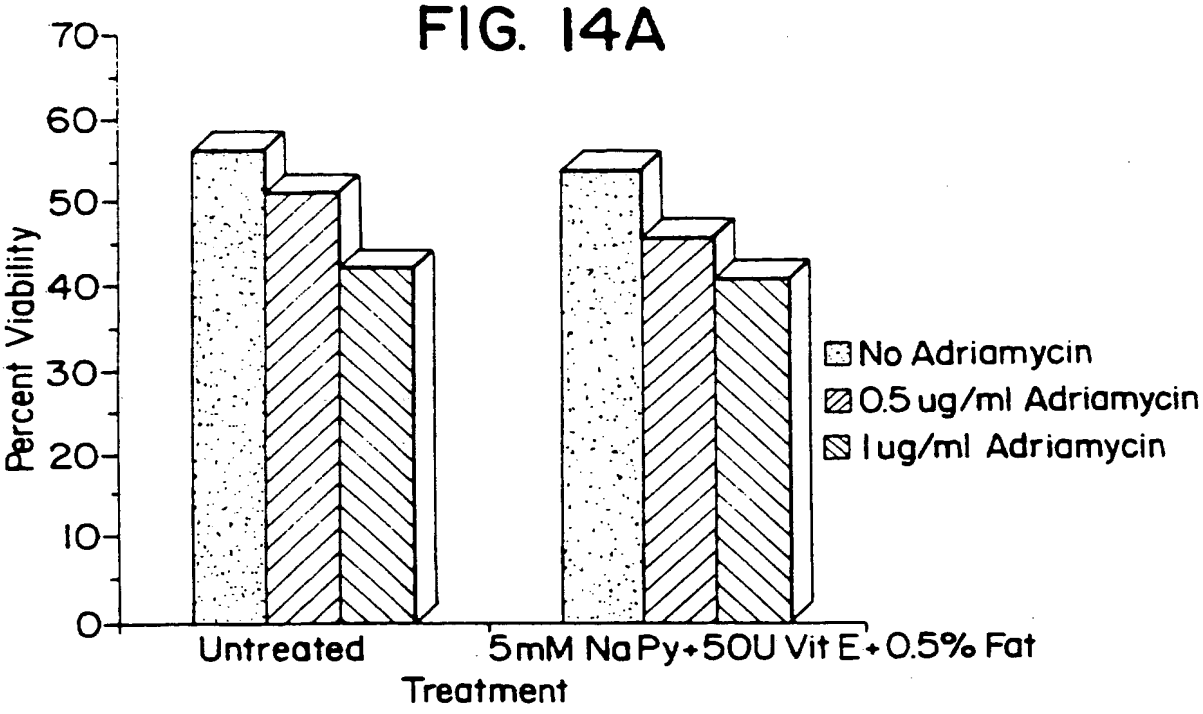


FIG. 14B

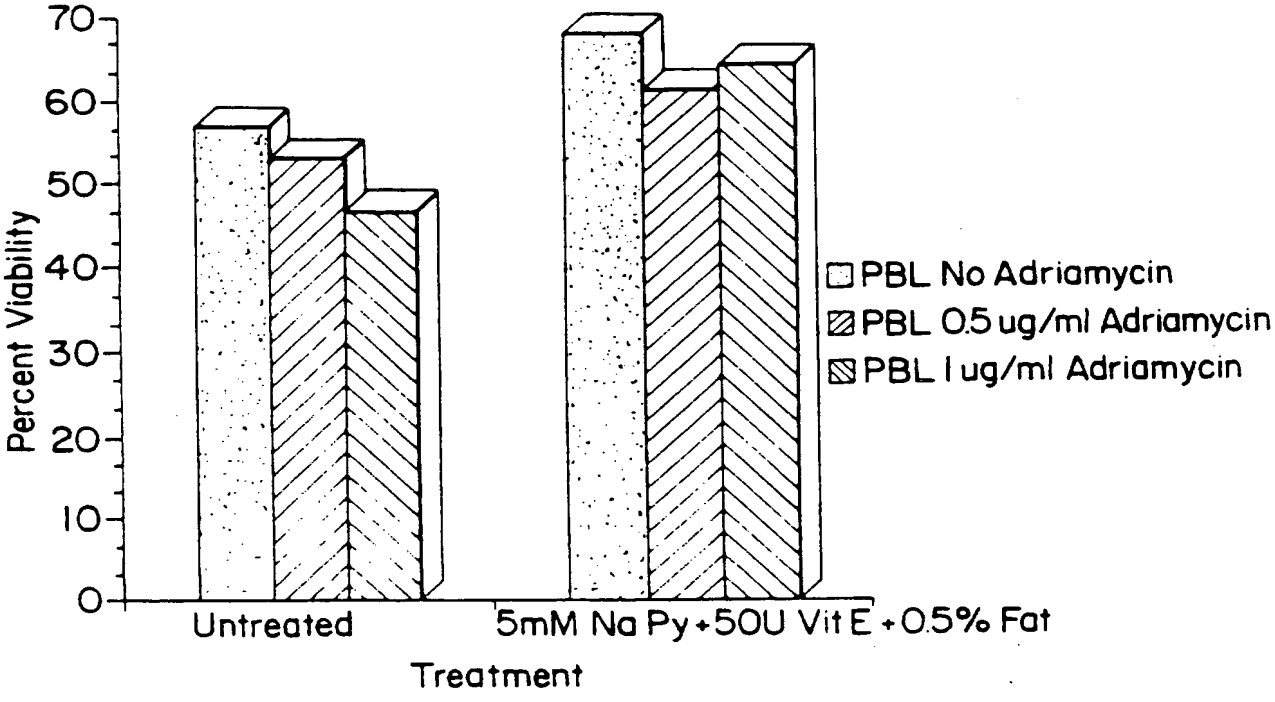


FIG. 15A

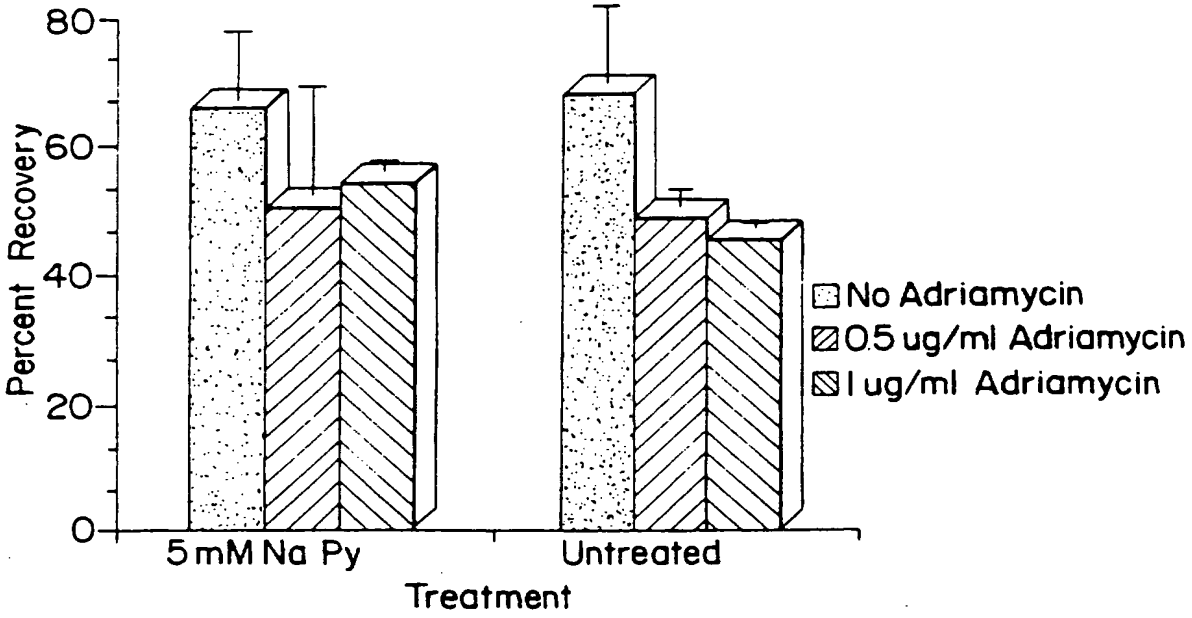


FIG. 15B

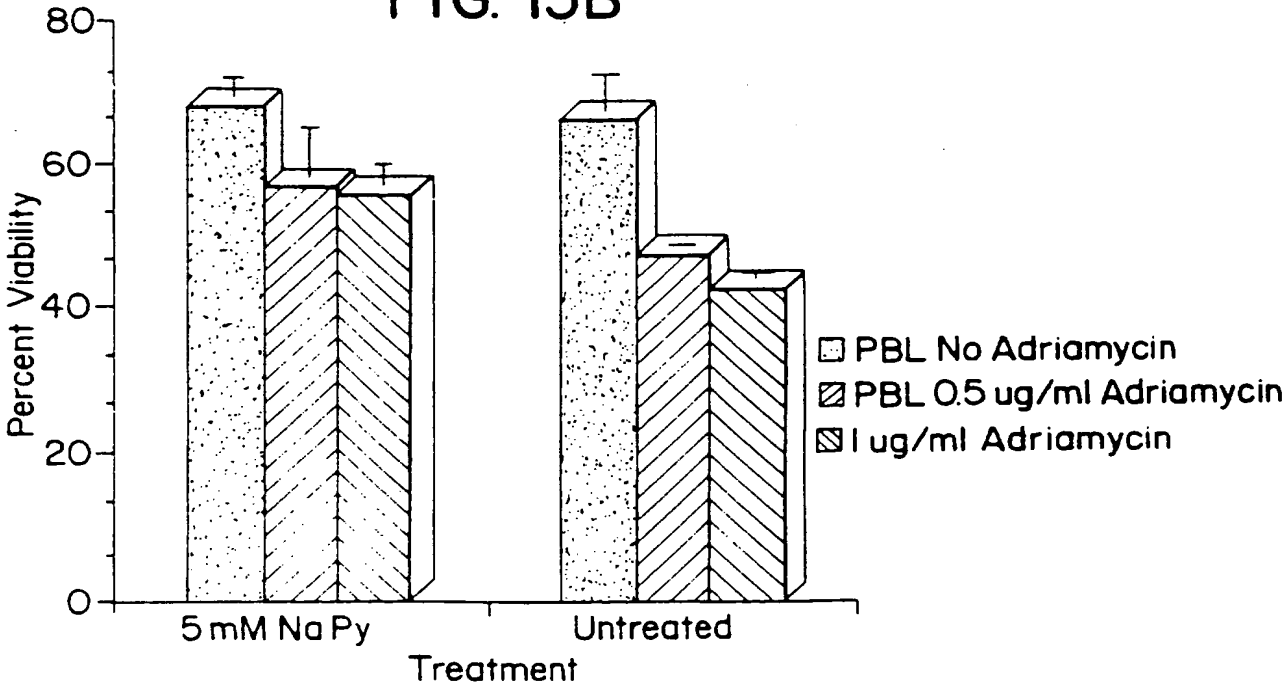


FIG. 16A

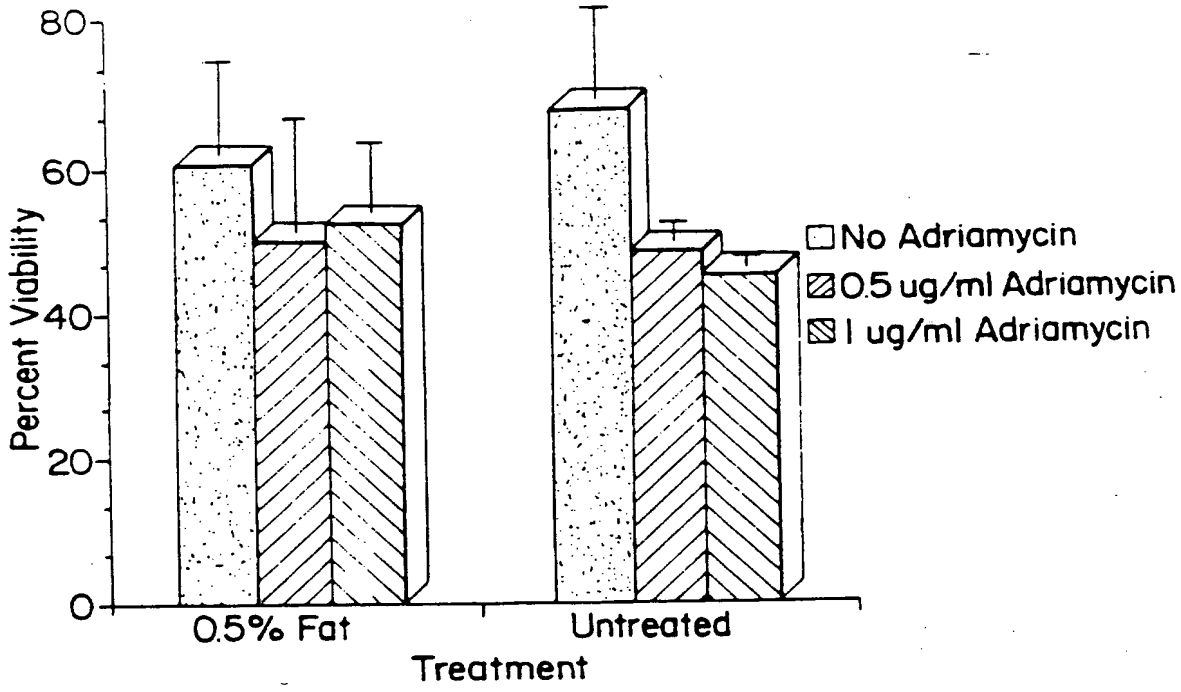


FIG. 16B

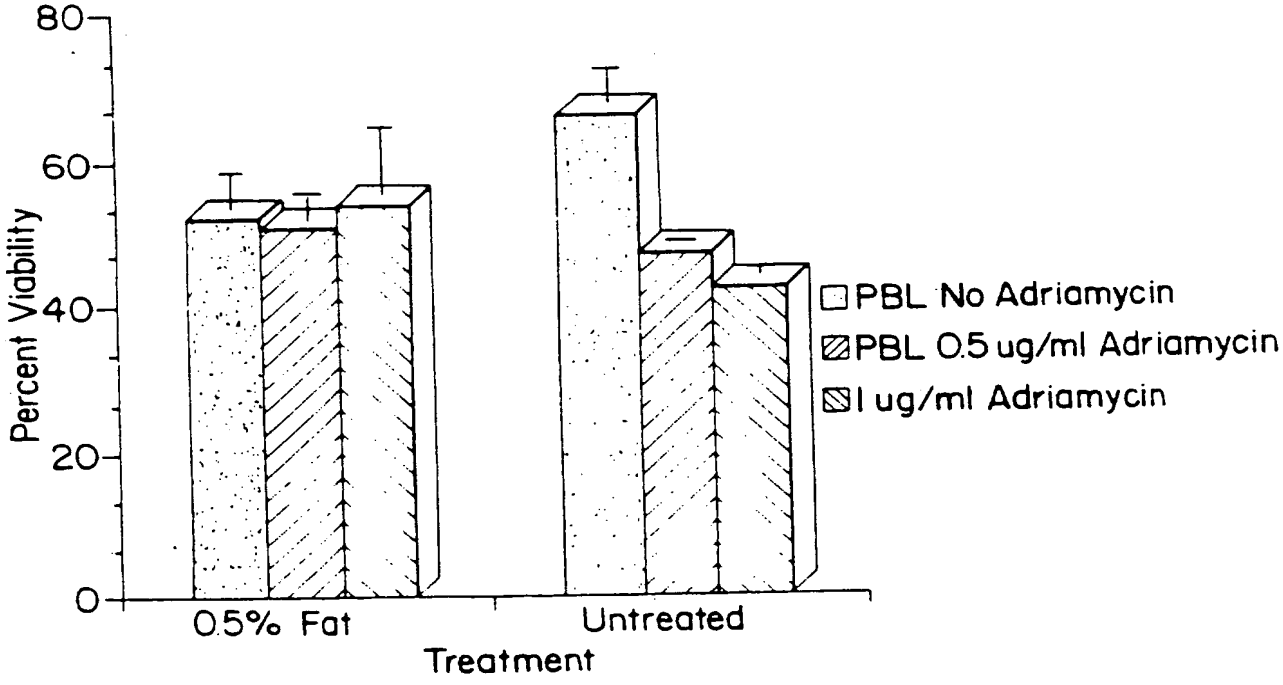


FIG. 17A

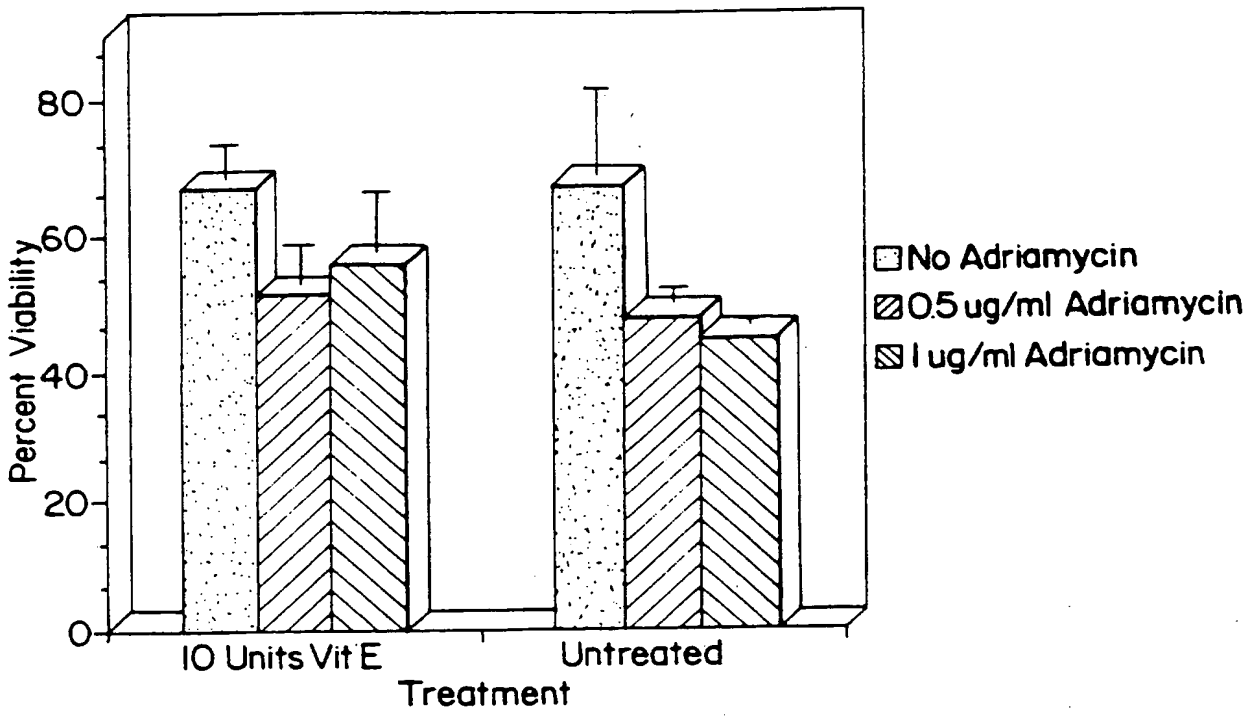


FIG. 17B

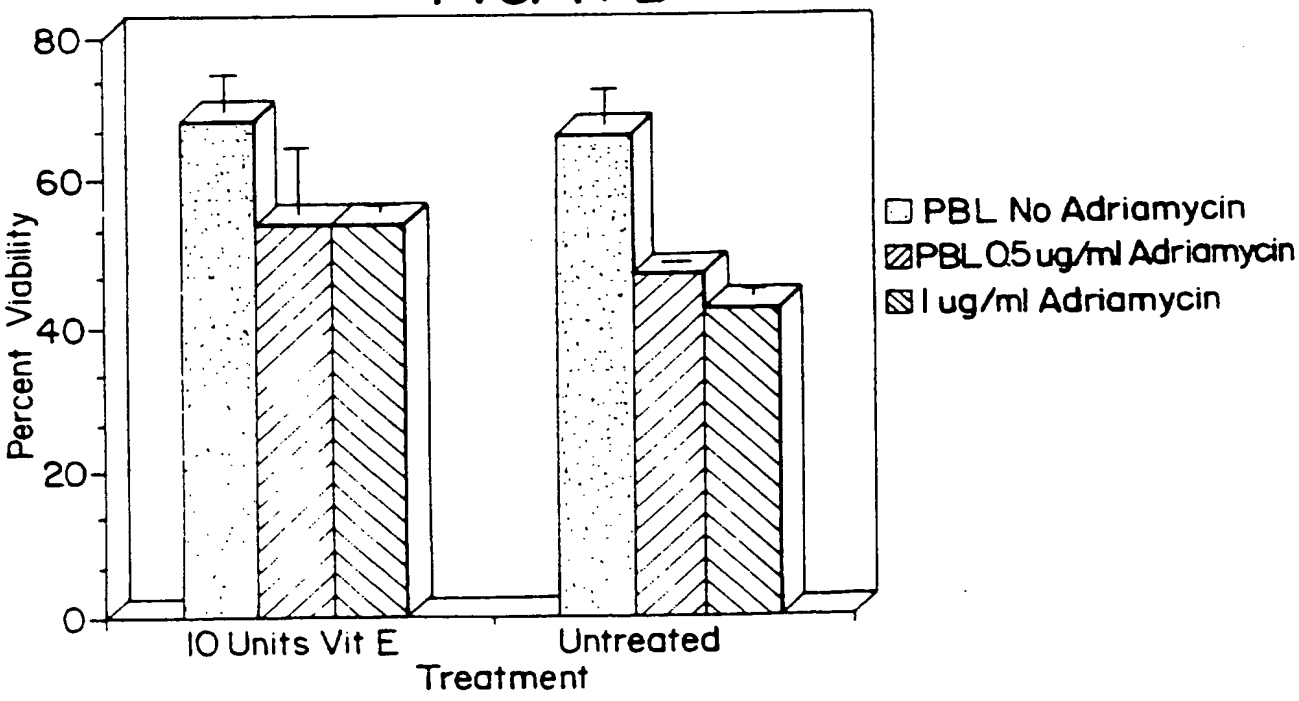


FIG. 18A

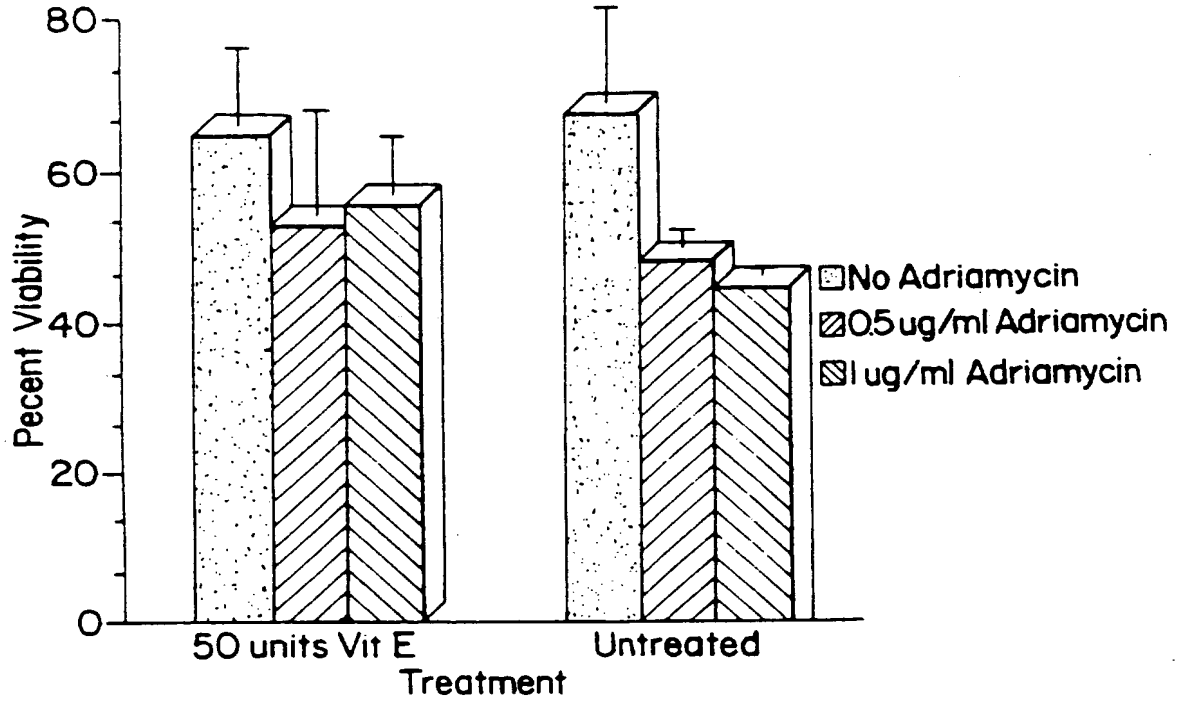


FIG. 18B

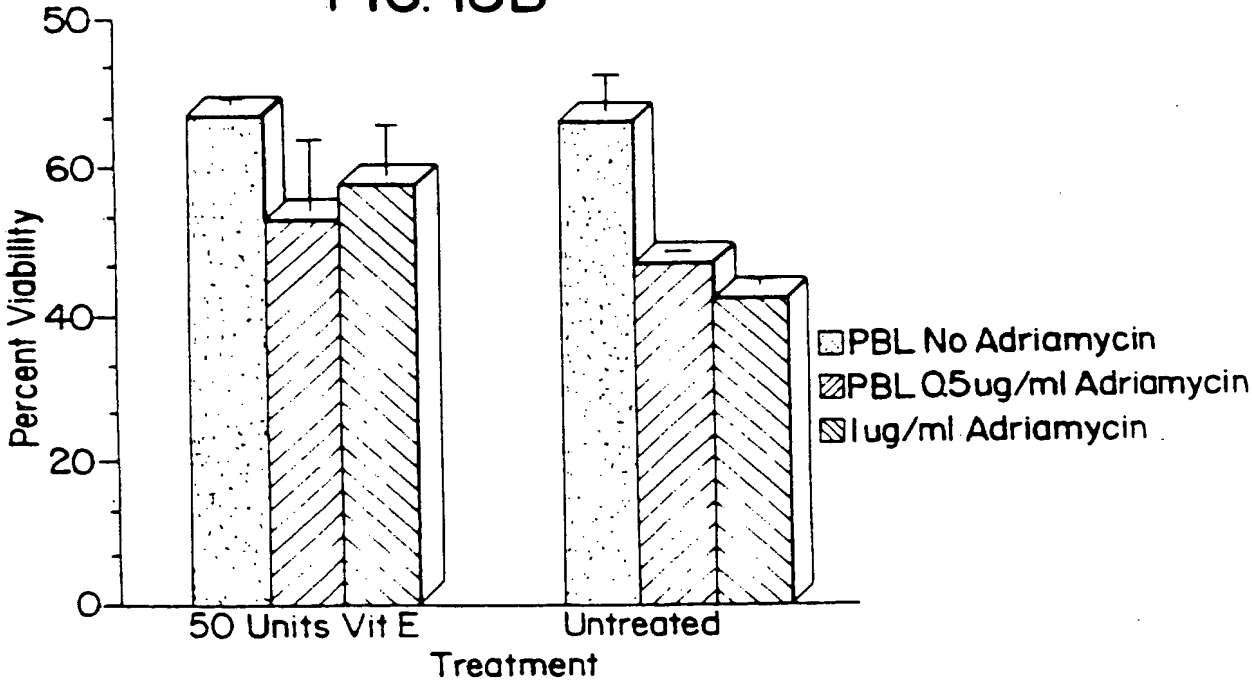


FIG. 19A

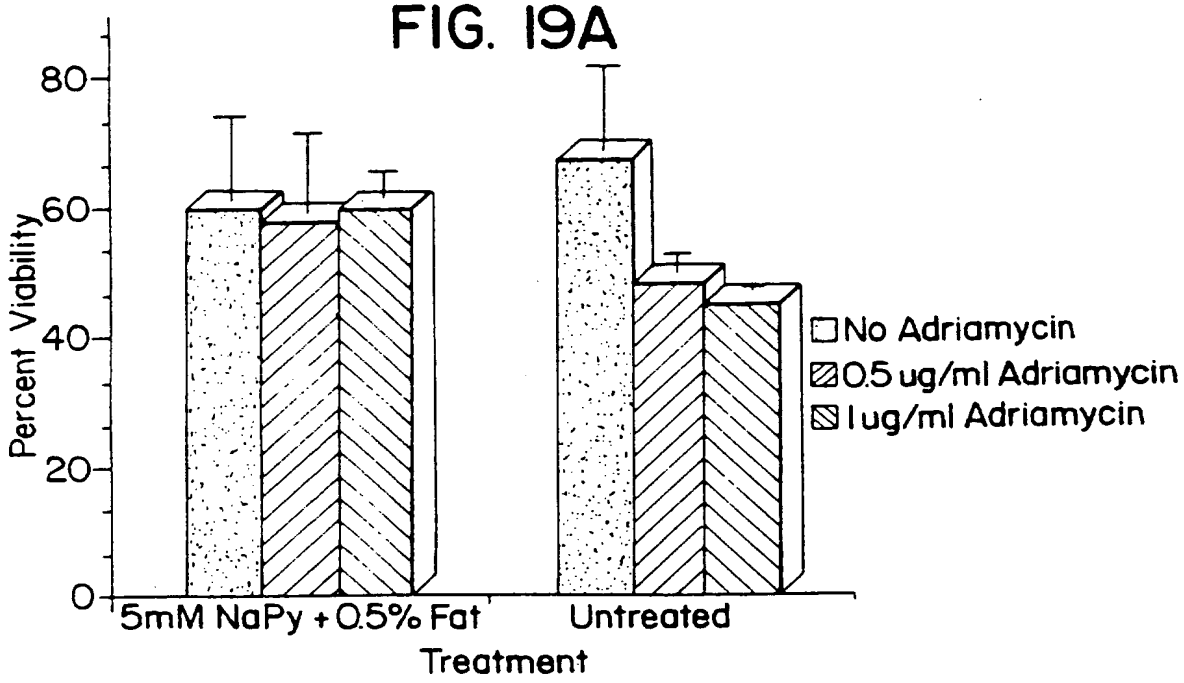


FIG. 19B

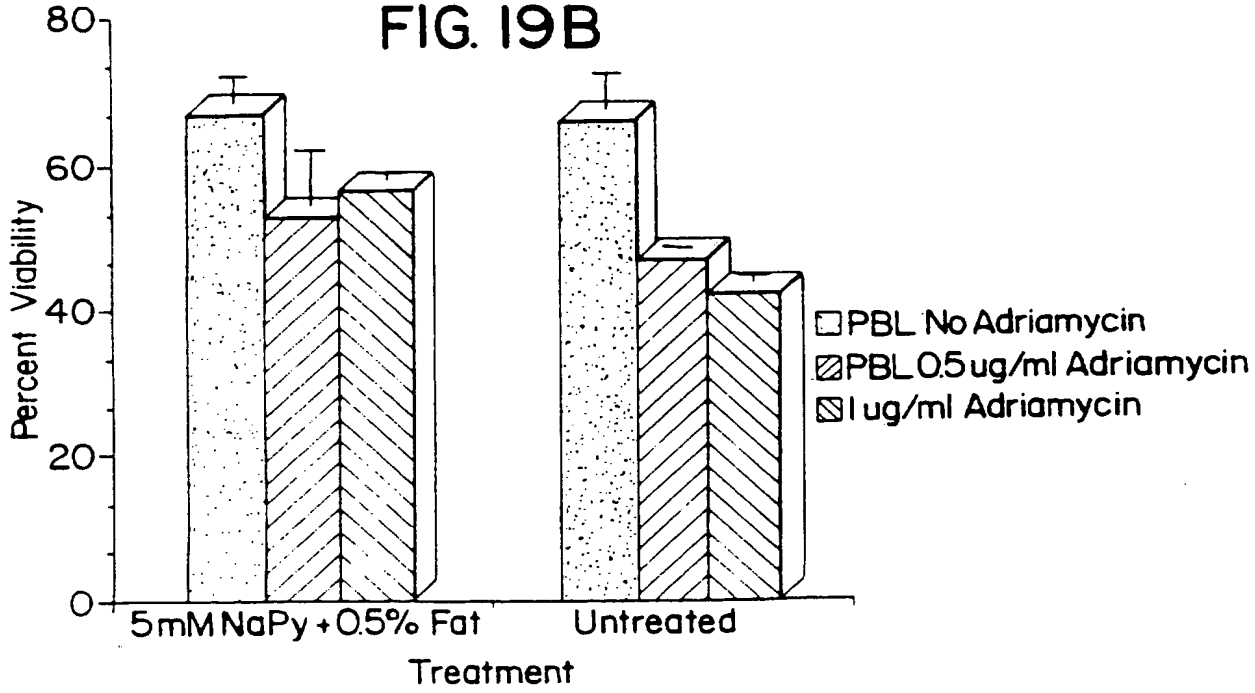


FIG. 20A

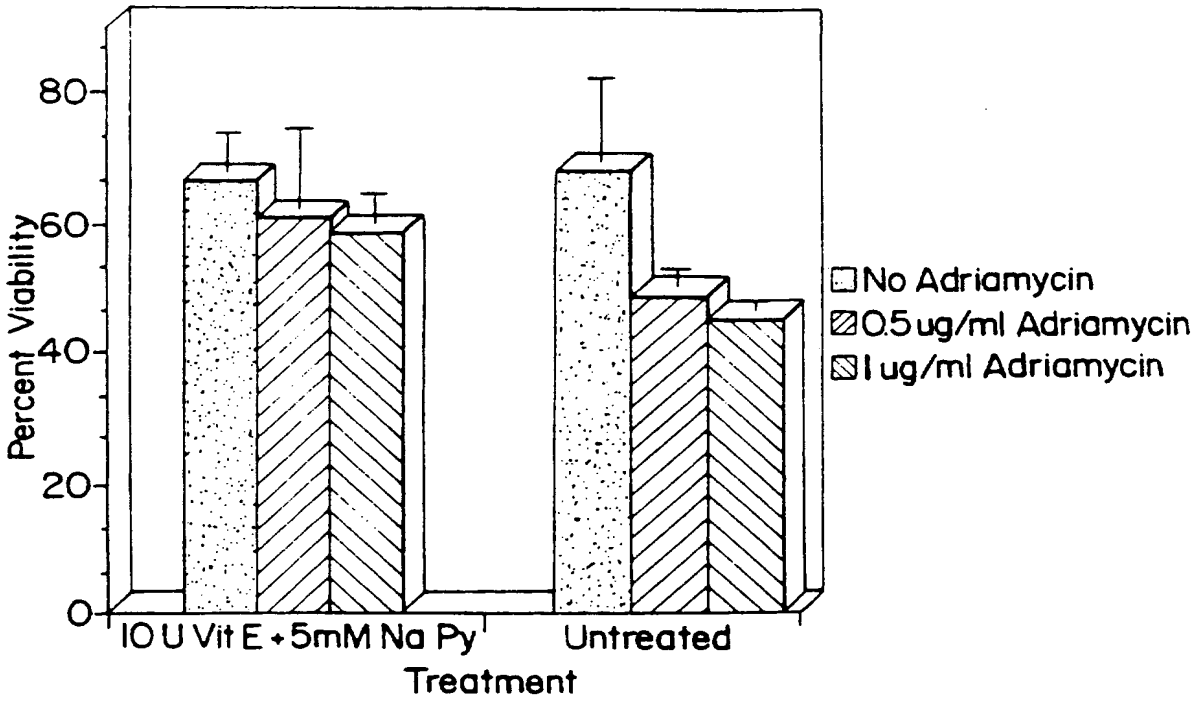


FIG. 20B

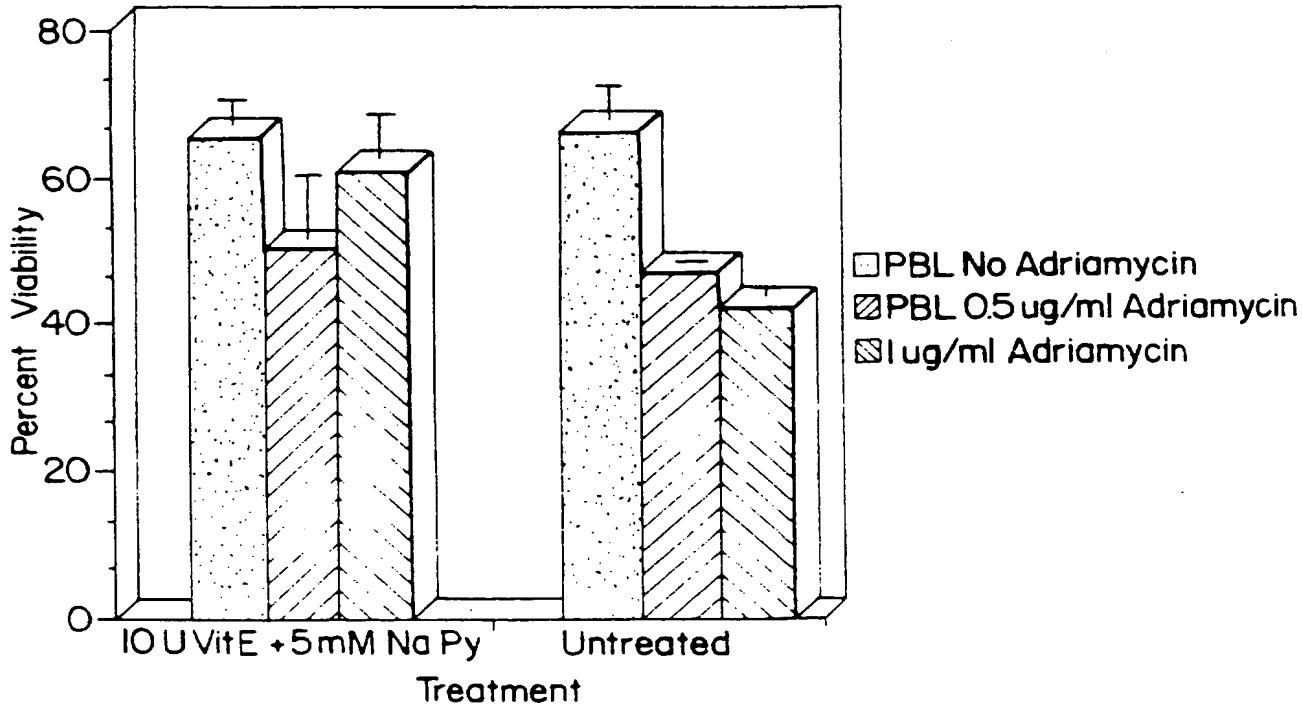


FIG. 21A

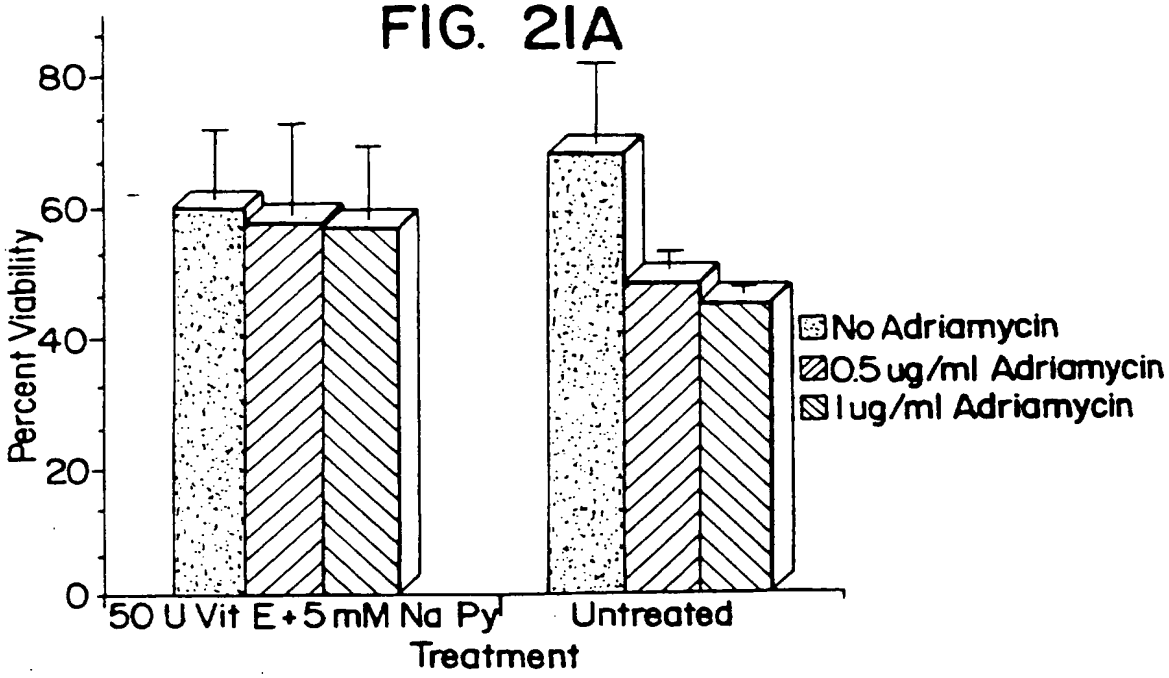


FIG. 21B

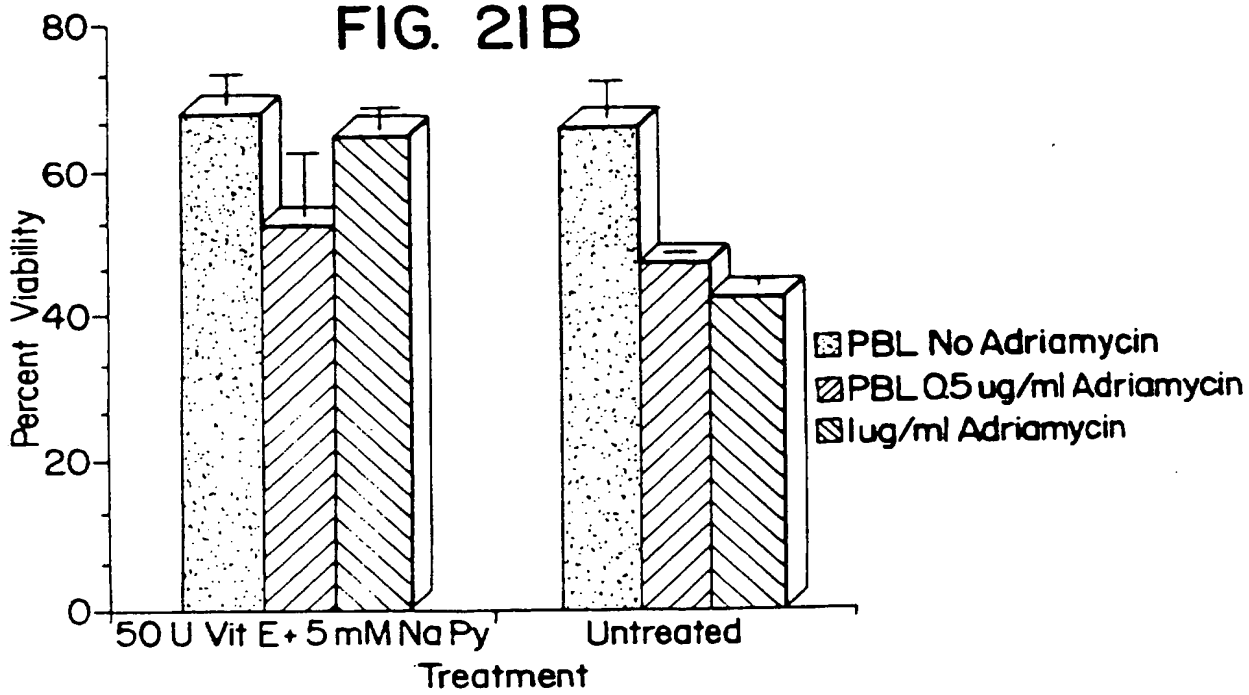


FIG. 22A

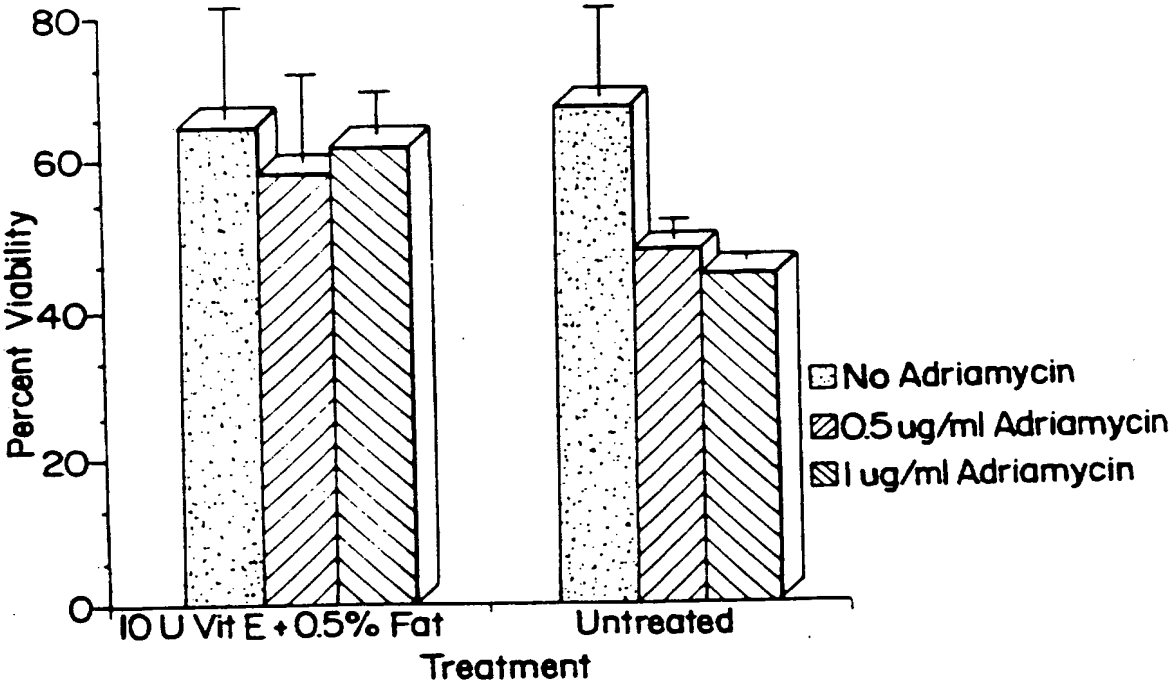


FIG. 22B

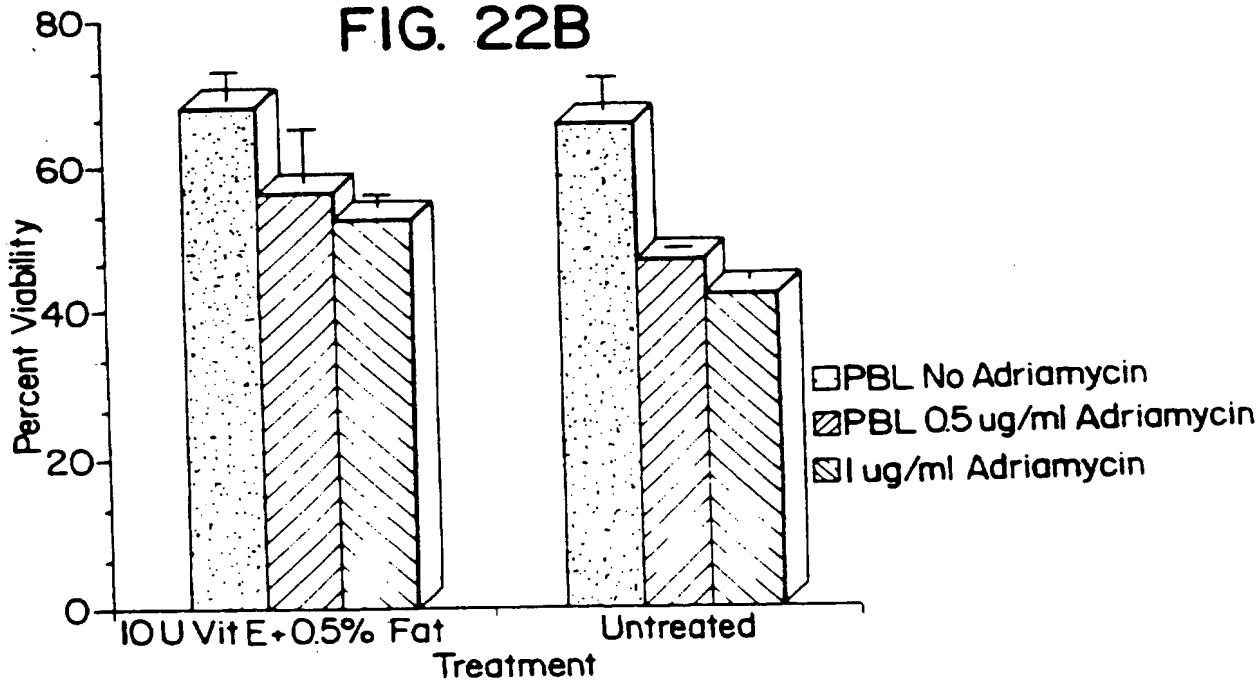


FIG. 23A

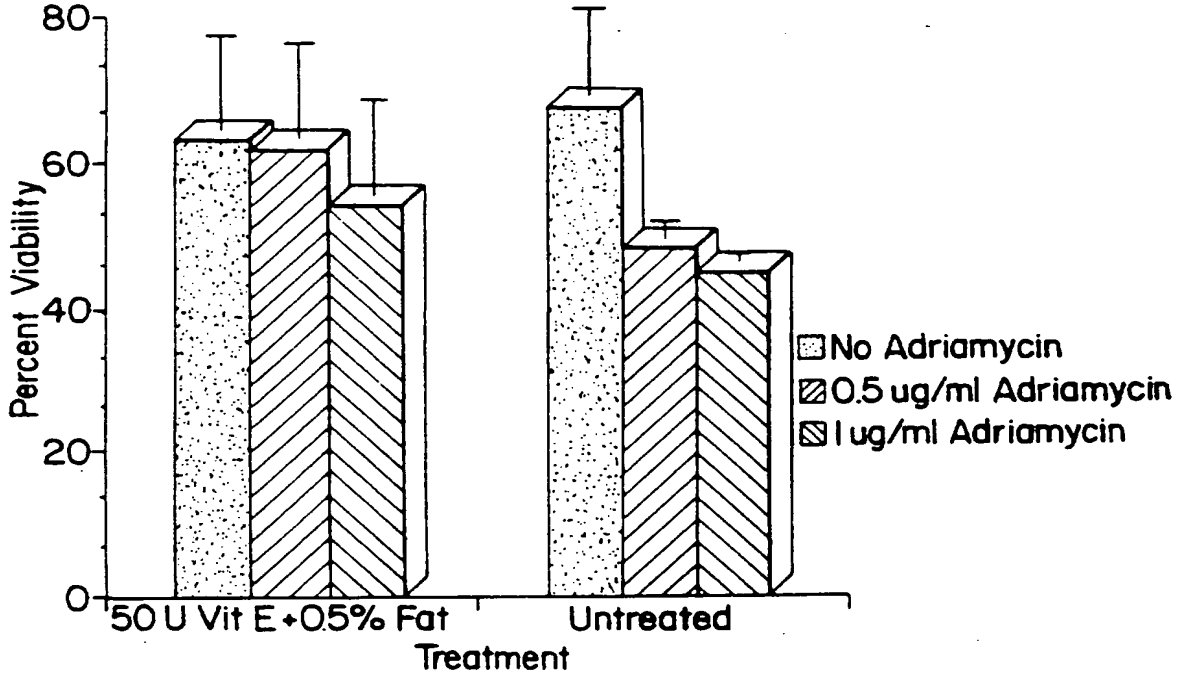


FIG. 23B

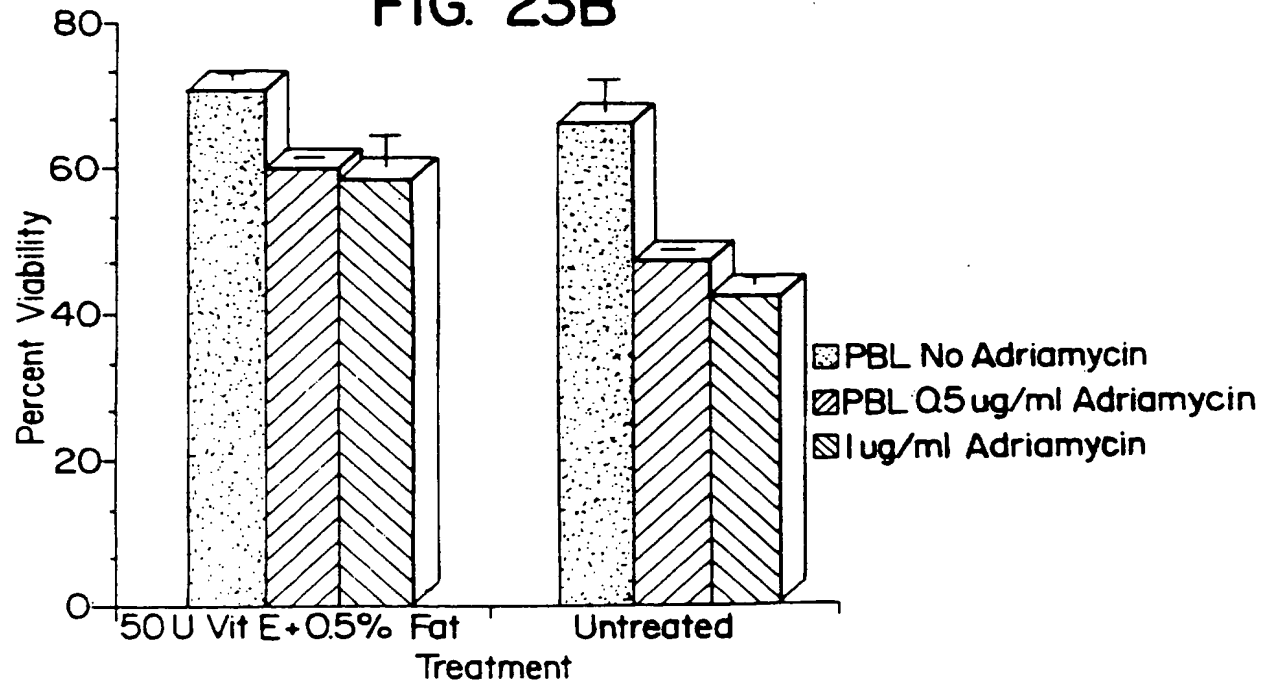


FIG. 24A

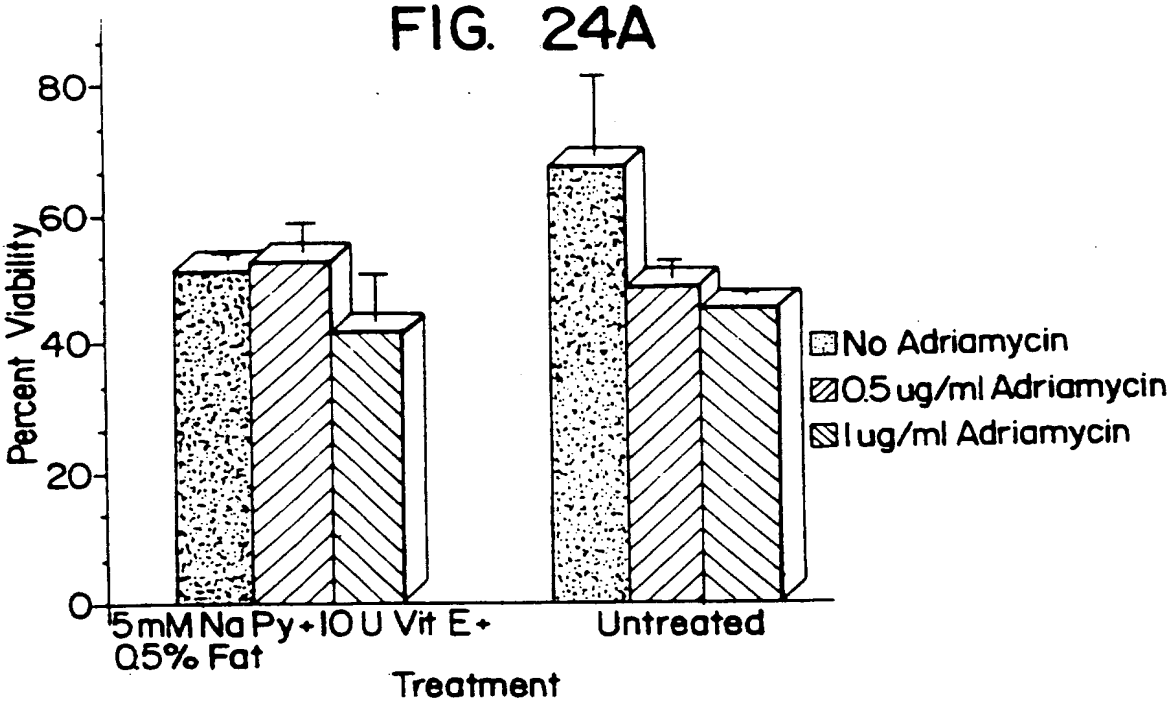


FIG. 24A

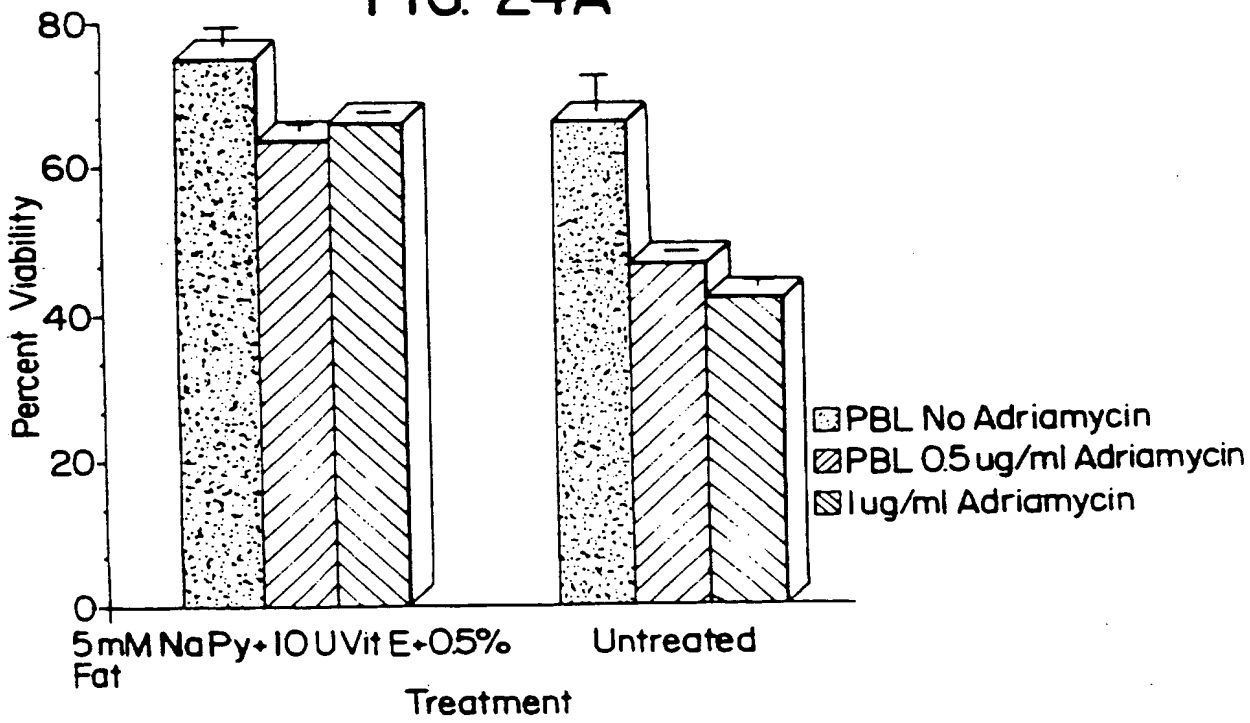


FIG. 25A

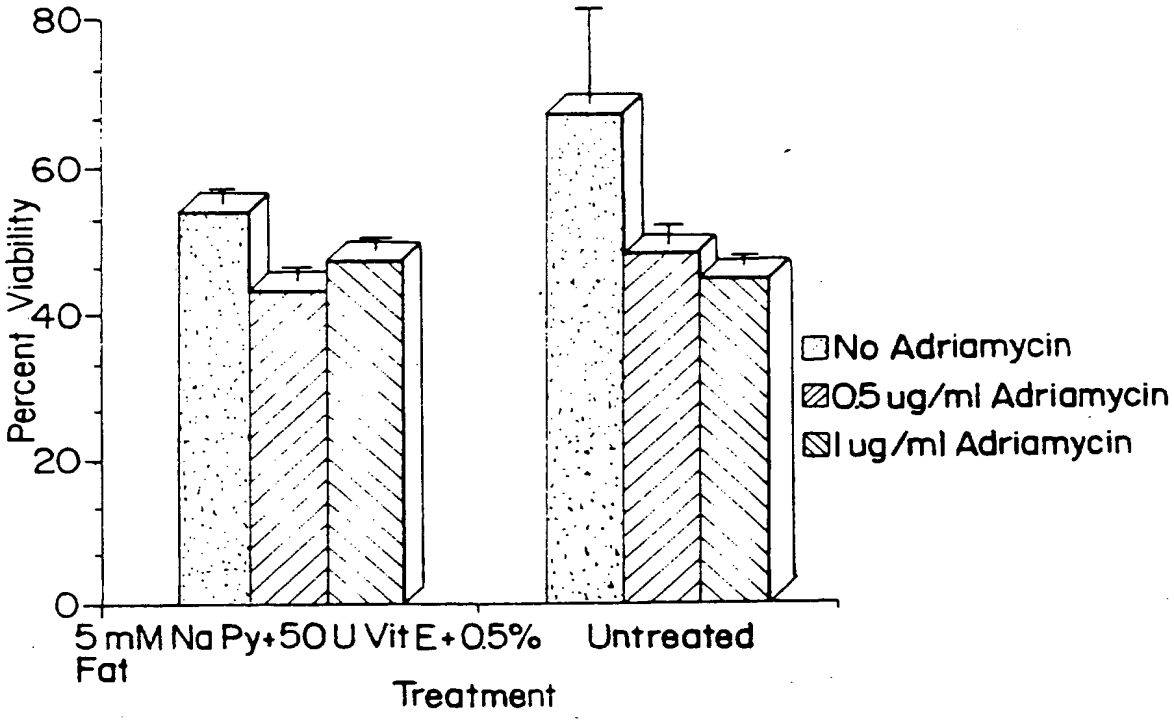
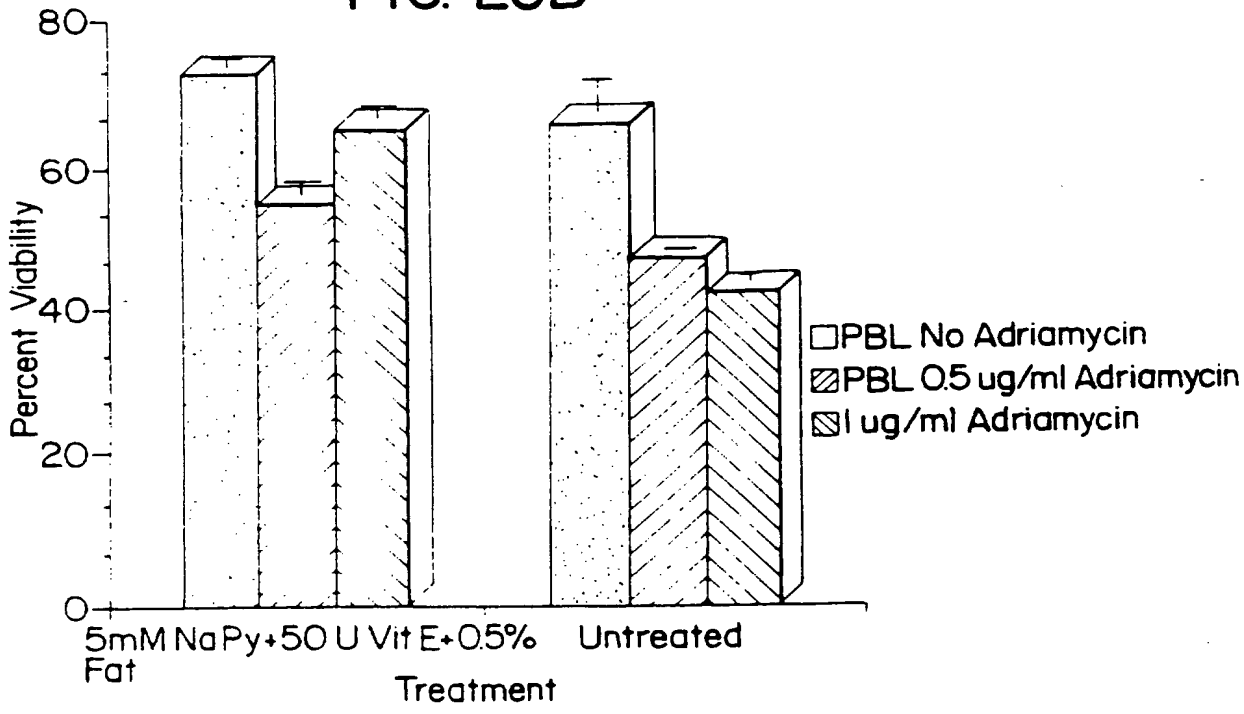


FIG. 25B



INTERNATIONAL SEARCH REPORT

International Application No PCT/US 93/00260

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl. 5 A61K31/19; A61K31/71; //(A61K31/19,31:20, 31:355, 31:375,31:07,31:015)

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
Int.Cl. 5	A61K

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category °	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No.[13]
P,X	WO,A,9 215 292 (WARNER-LAMBERT COMPANY) 17 September 1992 see abstract; claims	1-29
E	WO,A,9 310 776 (WARNER-LAMBERT COMPANY) 10 June 1993 see abstract; claims	1-29
X	WO,A,8 700 753 (R.L. LINDSTROM) 12 February 1987 see the whole document & US,A,4 696 917 cited in the application & US,A,4 725 586 cited in the application	1-7,20, 27

-/--

° Special categories of cited documents :[10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
30 JULY 1993	17. 08. 93
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	ORVIZ DIAZ P.

Form PCT/ISA/210 (second sheet) (January 1985)

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category °	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
X	EP,A,0 345 082 (K.K. HAYASHIBARA SEIBUTSU KAGAKU KENKYUJO) 6 December 1989 see the whole document, especially examples 8 and 18 and the claims	1-17,20, 21,27
X	J. ASSOC. OFF. ANAL. CHEM. vol. 74, no. 3, 1991, pages 522 - 525 A. MARTIN 'A resuscitation/selection system for rapid determination of Salmonella in foods.' see the whole document	1-17, 20-21,27
X	DATABASE WPI Section Ch, Week 7618, Derwent Publications Ltd., London, GB; Class B05, AN 76-33105X & JP,A,51 032 738 (M. NAKANISHI) see abstract	1-7,20, 27
Y	CANCER CHEMOTHER. PHARMACOL. vol. 5, no. 3, 1981, pages 175 - 179 M.H. COHEN 'Cure of advanced L1210 leukemia after correction of abnormal red blood cell deformability' cited in the application see the whole document	1-29
Y	IRCS MED. SCI. vol. 7, no. 10, 1979, page 505 S.M. AHMED 'Effects of ascorbic acid and various keto-acids on the glyoxalase enzyme system in mouse and rat liver.' see the whole document	1-29
Y	J. CLIN. INVEST. vol. 88, no. 6, 1991, pages 1886 - 1893 A.K. SALAHUDEEN 'Hydrogen peroxide-induced renal injury. A protective role for pyruvate in vitro and in vivo.' see the whole document	1-29
Y	FREE RADICAL RES. COMMUN. vol. 4, no. 5, 1988, pages 283 - 290 S.D. VARMA 'Peroxide damage to the eye lens in vitro prevention by pyruvate.' see the whole document	1-29

-/--

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category °	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
Y	J. EXP. MED. vol. 165, no. 2, 1987, pages 500 - 514 J. O'DONNELL-TORMEY 'Secretion of pyruvate. An antioxidant defense of mammalian cells.' see the whole document ---	1-29
Y	TOXICOL. LETT. vol. 28, no. 2-3, 1985, page 93-98 U. ANDRAE 'Pyruvate and related alpha-ketoacids protect mammalian cells in culture against hydrogen peroxide-induced cytotoxicity.' see the whole document -----	1-29

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/00260

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
REMARK: Although claims 24-26 are directed to a method of treatment of the human or animal body the search has been carried out and based on the alleged effects of the compositions.

2. [ ] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO.

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This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.
The members are as contained in the European Patent Office EDP file on
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information. 30/07/93

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For more details about this annex : see Official Journal of the European Patent Office, No. 12/82